BMJ Open

Total calcium should not be adjusted for albumin

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017703
Article Type:	Research
Date Submitted by the Author:	19-May-2017
Complete List of Authors:	Lian, Ingrid; St. Olavs hospital, Clinical Chemistry Åsberg , Arne; Sankt Olavs Hospital Universitetssykehuset i Trondheim, Department of Clinical Chemistry
Primary Subject Heading:	Diabetes and endocrinology
Secondary Subject Heading:	General practice / Family practice
Keywords:	Calcium & bone < DIABETES & ENDOCRINOLOGY, albumin, calcium

Total calcium should not be adjusted for albumin

Ingrid Alsos Lian and Arne Åsberg

Department of Clinical Chemistry, St. Olavs hospital, 7006 Trondheim, Norway Ingrid Alsos Lian Resident in Clinical Chemistry Department of Clinical Chemistry, St. Olavs hospital, 7006 Trondheim, Norway Arne Åsberg Consultant in Clinical Chemistry

Correspondence to: ingrid.alsos.lian@gmail.com

Word count: 251/2081 | Tables: 3 | Figures: 1 | References: 22

Abstract

Objectives: Albumin-adjusted total calcium is often used as a surrogate marker for free calcium to evaluate hypo- or hypercalcemia. Many adjustment-formulas based on simple linear regression models have been published, and continue to be used in spite of questionable diagnostic accuracy for calcium disturbances in various patient populations. We created local adjustment-formulas based on multiple linear regression, and used the estimated regression coefficients for albumin to test whether the diagnostic accuracy was improved compared to previously published formulas and unadjusted calcium.

Design: A retrospective hospital laboratory data study.

Data sources: The local hospital laboratory data system.

Setting: Norway, 2006-2015.

Participants: 6567 patients where total calcium, free calcium, creatinine, albumin and phosphate had been analysed in a single blood draw, including hospitalised patients and patients from outpatient clinics and general practice**.**

Main outcome measures: Diagnostic accuracy by Harrell's C and ROC curve analysis.

Results: The diagnostic accuracy was higher for unadjusted calcium than any albumin-adjusted calcium values based on various adjustment-formulas from the literature, in both patients with normal and high creatinine concentrations, according to both Harrell's C and ROC curve analysis. Even the locally constructed adjustment-formulas did not yield better diagnostic accuracy than unadjusted total calcium.

Conclusions: Our study shows that the diagnostic accuracy of *unadjusted* total calcium is superior to several commonly used adjustment-formulas, and we suggest that the use of such formulas should be abandoned in clinical practice. If the clinician does not trust total calcium to reflect the calcium status of the patient, free calcium should be measured.

Strengths and limitations of this study

- Albumin-adjusted total calcium is often used as a surrogate marker for free calcium, to evaluate hypo- or hypercalcemia. Many adjustment-formulas have been published, and continue to be used in spite of questionable diagnostic accuracy in various patient populations.
- The diagnostic accuracy was evaluated using free calcium as the gold standard, both as a dichotomous one (with ROC curve analysis) and as a continuous gold standard (with Harrell's C index), the latter providing less loss of information.
- This study includes a large group of both hospitalised and ambulant patients from a large regional hospital, representative of a broad spectrum of disease.

Introduction

Disturbances in calcium homeostasis is not uncommon in hospitalised patients [1, 2], although the exact prevalence in the general population is unknown. In plasma, only 50% of the calcium ions are free to exert biological effects, whereas the rest is bound to proteins, mostly albumin, and a few percent are bound in complexes with anions like lactate and citrate [3]. The concentration of free calcium ions is closely regulated, and patients with abnormal albumin concentrations may have a normal concentration of free calcium despite abnormal concentration of total calcium. Unfortunately, free calcium is not as easily measured as total calcium, the latter being a part of routine test panels of large automatic clinical chemistry instruments. Accordingly, clinicians often try to estimate the concentration of free calcium, using the concentration of total calcium and albumin. Usually this is indirectly done by calculating an albumin-adjusted calcium value, i.e. the clinician asks *"What is the patient's concentration of total calcium if the albumin concentration is normal?"* Changes in the concentration of free calcium due to acidemia or alkalemia are disregarded in these cases. Several adjustment-formulas have been used [4-7], and continue to be so [8], in spite of their rather questionable diagnostic accuracy [9], which may be worse than that of unadjusted calcium in certain populations [10].

The reason why the adjustment-formulas perform so poorly is not completely clear. Some argue that a certain formula is only valid for specific patient populations [10], others that a certain formula may only be valid for certain analytical methods [11]. We hypothesise a more fundamental flaw – that the adjustment-formulas are based on wrongly formulated regression models. These formulas are estimated from patient populations with a range of total calcium and albumin concentrations, where the investigators have regressed the concentration of total calcium against albumin, using least squares regression [4, 5]. The regression coefficient of albumin, usually in the range of 0.018-0.025 [6], then tells how much the total concentration of calcium is expected to change for one unit change in

albumin concentration, comparing two hypothetical patients with different albumin concentrations. However, what the clinician really wants to know is how much the total concentration of calcium is expected to change for one unit change in albumin concentration *when the patient's condition is otherwise unchanged, specifically when the concentration of free calcium is unchanged*. Therefore, we ought to regress the concentration of total calcium against albumin *and* free calcium, sex, age or whatever explanatory variable is relevant, so that we can estimate the expected change in the total concentration of calcium for one unit change in albumin concentration *per se*, holding the other variables constant. Then the interpretation of the albumin coefficient gets in line with the clinical use. The purpose of this study was (i) to estimate regression coefficients for albumin from regression models with and without the concentration of free calcium and other relevant explanatory variables, and (ii) to test whether these different regression coefficients yielded albumin-adjusted calcium values of different diagnostic accuracy.

Material and methods

Material

Laboratory data was collected retrospectively, from January 1st 2006 to September 18th 2015 from 6567 patients, where analysis of total calcium, free calcium, creatinine, albumin and phosphate had been performed in blood samples from the same blood draw. Only a single data set (the oldest), from each patient was included. We included data from both hospitalised patients and patients from outpatient clinics and general practice. All samples were analysed at our laboratory at St.Olavs hospital, Trondheim, Norway.

Laboratory analyses

Albumin, total calcium, creatinine and phosphate were assayed by colorimetric methods on fully automated Modular P800 or Roche Cobas 6000 c501 instruments (Roche Diagnostics, Mannheim, Germany). The bromcreosol green (BCG) method was used for albumin. The concentration of free calcium was measured by an ionselective electrode mounted in an automated blood gas analyser (ABL 725, Diamond Diagnostics, Holliston, MA, USA), and standardised at pH 7.40.

Reference ranges

Reference ranges for total calcium is 2.15-2.51 mmol/L [12], 1.18-1.32 mmol/L for free calcium, whereas our laboratory use age- and sex specific reference ranges for albumin and creatinine [12-14].

Patient involvement

There was no direct patient involvement in the development, design or conduct of the study.

Statistical analysis

The dataset was divided into subgroups with creatinine concentrations below or above the upper reference range, as others have found different albumin coefficients in individuals with renal failure compared to individuals with normal renal function [15]. In addition, we divided the dataset according to albumin concentrations below or above 27 g/L, as plots of total calcium against albumin indicated nonlinearity overall, but linearity below and above 27 g/L. Altogether, this procedure resulted in four subgroups. We created albumin-adjustment formulas for these subgroups: Adjusted calcium = calcium + coefficient × (40 - albumin), where the group-specific albumin coefficients were estimated using multiple linear regression models with total calcium as the dependent variable and free calcium, albumin, phosphate, creatinine, sex and age as the explanatory variables. We used backwards elimination until all remaining explanatory variables were statistically significant.

The diagnostic accuracy of albumin-adjusted calcium calculated from the local formulas was compared to that of unadjusted total calcium and five other commonly used adjustment-formulas, taken from literature [4-7, 16]. First, we used free calcium as a dichotomous gold standard and compared the diagnostic accuracies with receiver operating characteristic (ROC) curve analysis, where the patients were classified as hypocalcemic or not, and hypercalcemic or not, according to the reference range for free calcium. Second, we used free calcium as a continuous gold standard with Harrell's C index as a measure of diagnostic accuracy. This index is directly related to the area under the ROC curve [17]. Both measures can take on values from 0.5 (no diagnostic accuracy) to 1.0 (perfect diagnostic accuracy) but Harrell's C uses free calcium as a continuous gold standard. To test whether albumin-adjusted calcium correlated better with the reference ranges than unadjusted calcium, we used locally weighted trend (lowess) lines in a plot of free calcium against total calcium. Laboratory data was extracted using SAS (version 9.2 for Windows, SAS Institute, NC, USA) and analysed using STATA (version 13.1 for Windows, StataCorp LP, TX, USA). $P < 0.05$ was considered statistically significant.

Results

Study population

A total of 6567 samples were collected, from 3895 women (59.3%) and 2672 men (40.7%). The median age was 49 years, ranging from 21 days to 96 years. The mean concentration of total calcium was 2.30 mmol/L (range 1.18-5.40), whereas the mean concentration of free calcium was 1.22 mmol/L (range 0.52-3.10). Using free calcium as the reference, 5312 patients (81%) were normocalcaemic, 855 (13%) had hypocalcemia, and 400 (6%) had hypercalcemia. The concentration of albumin ranged from 14.3 to 51.0 g/L, with a mean of 39.6 g/L. A total of 877 (13%) and 120 (1.8%) had albumin concentration below or above the reference range for age and sex, respectively, and 1467 patients (22%) had creatinine concentration above the upper reference range.

Multiple linear regression analyses

For patients with normal creatinine concentrations, the regression coefficient for albumin was 0.0125 in the overall group; in the subgroup of patients with albumin <27 g/L, the regression coefficient was 0.0145, whereas for patients with albumin ≥27 g/L, the regression coefficient was 0.012 (Table 1). For patients with high concentrations of creatinine (above the age-specific reference range), the coefficient was 0.0121 regardless of albumin status, whereas it was 0.0084 in patients with albumin<27 g/L, and 0.0114 in patients with albumin ≥27 g/L. Comparing the patient population with albumin <27 g/L, the albumin coefficient was 58% higher for patients with normal creatinine concentration compared to those with high creatinine concentration.

Analyses of diagnostic accuracy

Harrell's C index was higher for unadjusted calcium than any albumin-adjusted calcium values

based on various adjustment-formulas from the literature, in both patients with normal and high creatinine concentrations (Table 2). In addition, the area under the ROC curve for hypocalcemia was higher for unadjusted calcium than that of any albumin-adjusted calcium values derived from formulas from literature (Table 3). Even the locally constructed adjustment-formulas, constructed and tested in the same dataset, did not yield better diagnostic accuracy than unadjusted total calcium. A superior diagnostic accuracy for unadjusted calcium is also shown in Figure 1, where unadjusted and albumin-adjusted total calcium values according to the BMJ-formula (albumin-adjusted calcium values according to the formula suggested in BMJ in 1977 [6]) is plotted against free calcium for patients with hypoalbuminemia. The trend line of unadjusted calcium is closer to the intersections of lines of the reference limits than the trend line of albumin-adjusted calcium.

Discussion

We found that the regression coefficient for albumin varied greatly, depending on which subpopulation we studied, especially between patients with low concentration of albumin and either high or normal concentrations of creatinine. This indicates that the use of a single adjustment-formula across different patient populations will give misleading results. By using Harrell's C as a measure of agreement between total calcium (both adjusted and unadjusted) and free calcium (used as gold standard for calcemia-status), we found that the diagnostic accuracy of albumin-adjusted calcium based on formulas from the literature was inferior to that of unadjusted calcium, in both patients with normal and high concentrations of creatinine. This was also shown by a ROC curve analysis, where the diagnostic accuracy of albumin-adjusted calcium was inferior to unadjusted total calcium in diagnosis of hypocalcemia. Even our locally created formulas, which would be expected to yield good results, as they were estimated from correctly formulated regression models and tested in the same dataset, did not show better diagnostic accuracy than that of unadjusted calcium. Lastly, the reference limits of total calcium were better suited for unadjusted than for albumin-adjusted calcium (Figure 1).

The diagnostic accuracy of albumin-adjustment formulas has previously been questioned. Already in 1978, Ladenson et al. [9] compared 13 different albumin-adjustment formulas in blood samples from 375 hospitalised patients and 53 controls, and found that none of the formulas were consistently superior to unadjusted total calcium in predicting the correct calcium status. We have found no evidence in the literature supporting that adjusted calcium is superior to unadjusted calcium in this aspect. Our study includes a relatively large group of both hospitalised and ambulant patients from a large regional hospital, including both the critically ill and the more "normal" patient, representative of a broad spectrum of disease. Compared to Ladenson et al. [9], our findings are strengthened by the large study population. We have evaluated the diagnostic accuracy using

free calcium as the gold standard, both as a continuous gold standard (with Harrell's C index) and as a dichotomous one (with ROC curve analysis), because some information may be lost when a continuous variable is dichotomised. Both methods demonstrated that unadjusted calcium was the most accurate indicator of free calcium status. This has also been shown by others, who have used other measures as the Pearson correlation coefficient between free and adjusted calcium [9, 18], the intraclass correlation coefficient [10] or the number of discordant results of calcium status classification [9, 18].

A proposed weakness of these adjustment-formulas has been that they assume that an average regression coefficient is applicable to all patients. Especially for patients with chronic kidney disease, it has been shown that albumin-adjusted calcium has been unreliable in predicting the free calcium status [18, 19]. Even so, adjustment-formulas continue to be used and recommended in general clinical practice [8, 20]. Even clinical guidelines in renal disease promote the use of albumin-adjusted calcium [21]. In a position paper from 2006, the Kidney Disease: Improving Global Outcomes (KDIGO) acknowledged that calcium status is best monitored by measuring free calcium, but they also stated that if total calcium was used instead, it should be adjusted for low concentrations of albumin [22].

Conclusion

In conclusion, we find that the diagnostic accuracy of unadjusted calcium is superior to that of albumin-adjusted total calcium based on formulas from literature, and even to that of locally constructed adjustment-formulas especially adapted to our dataset. At least one paper has previously reported similar findings to those of ours, almost 40 years ago [9]. Despite this, albumin-adjustment of calcium continues to be used in general clinical practice. Albumin-adjusted total calcium is a poor tool for identifying disturbances in calcium homeostasis, and comparing albumin-adjusted calcium values against the normal reference limits of total calcium may lead to inappropriate diagnoses. If the clinician does not trust total calcium to reflect the calcium status of the patient, free calcium should be measured, as that analysis is now widely available.

No competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical consideration

The study was carried out in full accordance with the ethical principles of the Declaration of Helsinki. According to the Norwegian Health Research Act, this type of project which uses anonymous information from local health registers, is not required to be evaluated by the Norwegian Regional Ethical Committee (REC).

Research funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Acknowledgments

None declared.

Data sharing

The full dataset is available upon request from the corresponding author (ingrid.alsos.lian@gmail.com). Informed consent for data sharing was not obtained, but the presented data are anonymised and risk of identification is low.

Contributors

IAL and AÅ both contributed to the acquisition, analysis, and interpretation of data for the work, the drafting the work or revising it critically for important intellectual content. AÅ was the main contributor in the conception and design of the work. IAL is the guarantor.

Table 1. Results from the multiple linear regression models

Normal creatinine	**Significant variables**	**Albumin coefficient [95% CI[**	**Adjusted R^2**
All patients (*n*=5100)	Albumin, free calcium, creatinine, phosphate, age	0.0125 [0.0119-0.0130]	0.75
Albumin <27 g/L (*n*=77)	Albumin, free calcium	0.0145 [0.0077-0.0211]	0.91
Albumin ≥27 g/L (*n*=5023)	Albumin, free calcium, creatinine, phosphate, age	0.0120 [0.0113-0.0127]	0.71
High creatinine			
All patients (*n*=1467)	Albumin, free calcium, creatinine, phosphate, age	0.0121 [0.0110-0.0131]	0.80
Albumin <27 g/L (*n*=104)	Albumin, free calcium, creatinine	0.0084 [0.0023-0.0144]	0.92
Albumin ≥27 g/L (*n*=1363)	Albumin, free calcium, creatinine, phosphate, age, sex	0.0114 [0.0099-0.0128]	0.76

Different albumin coefficients in patients with normal or high concentration of creatinine, and low or normal/high concentration of albumin. R^2 shows the percentage of the variable variation that are explained by the model. $P < 0.05$ was considered statistically significant.

Table 2. Agreement between total calcium and free calcium as measured by Harrell's C index [95% confidence interval] in patients with normal and high concentrations of creatinine

Adjustment-formula	Normal creatinine (*n*=5100)	High creatinine (*n*=1467)
None	0.74 [0.74-0.75]	0.79 [0.77-0.80]
Local *	0.74 [0.73-0.75]	0.77 [0.76-0.79]
Orrell [4]	0.73 [0.72-0.74]	0.74 [0.72-0.76]
BMJ [6]	0.72 [0.71-0.73]	0.73 [0.71-0.74]
Thode [16]	0.72 [0.71-0.73]	0.72 [0.70-0.74]
Berry [7]	0.71 [0.70-0.72]	0.71 [0.69-0.72]
Payne [5]	0.70 [0.69-0.71]	0.69 [0.68-0.71]

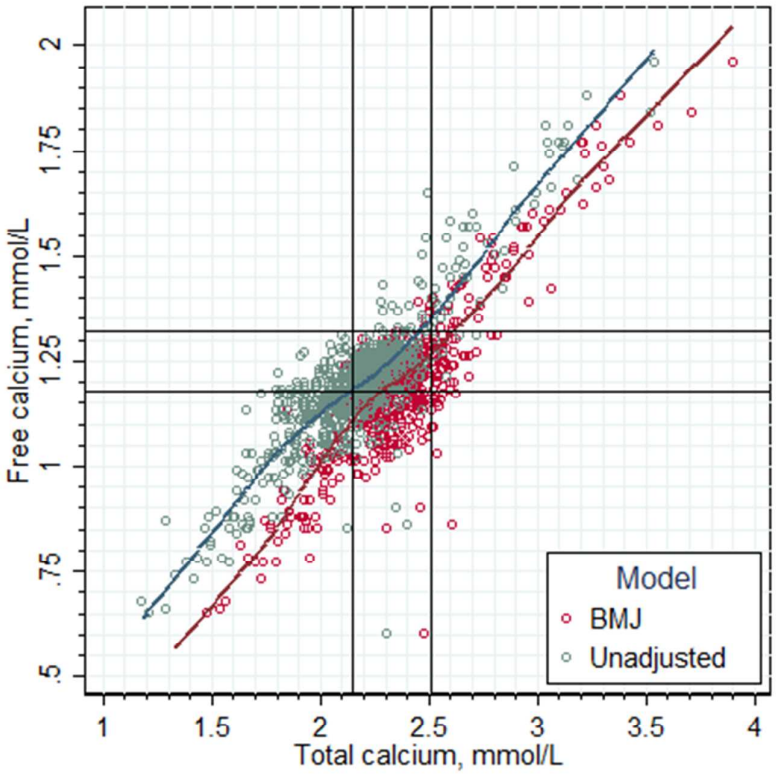
Using Harrell's C index to evaluate diagnostic accuracy of unadjusted and albumin-adjusted calcium according to various formulas taken from literature, where the concentration of free calcium is the gold standard. *A local adjustment-formula was constructed using four subgroup-specific albumin coefficients.

Table 3. Receiver operating characteristic curve analysis of hypo- and hypercalcemia in 6567 patients

Adjustment-formula	Hypocalcemia, free calcium <1,18 mmol/L [95% CI]	Hypercalcemia, free calcium >1,32 mmol/L [95% CI]
None	0.86 [0.85-0.87]	0.97 [0.96-0.98]
Local *	0.82 [0.81-0.84]	0.98 [0.97-0.98]
Orrell [4]	0.77 [0.75-0.79]	0.97 [0.97-0.98]
BMJ [6]	0.74 [0.72-0.76]	0.97 [0.96-0.98]
Thode [16]	0.74 [0.72-0.76]	0.97 [0.96-0.98]
Berry [7]	0.74 [0.69-0.73]	0.97 [0.96-0.98]
Payne [5]	0.69 [0.67-0.71]	0.96 [0.96-0.97]

Receiver operating characteristic curve analysis of hypo- and hypercalcemia, using the concentration of free calcium as the gold standard. The diagnostic accuracy of albumin-adjusted calcium values derived from various formulas taken from literature, were all inferior compared to unadjusted calcium for hypocalcemia. *A local adjustment-formula was constructed using four subgroup-specific albumin coefficients.

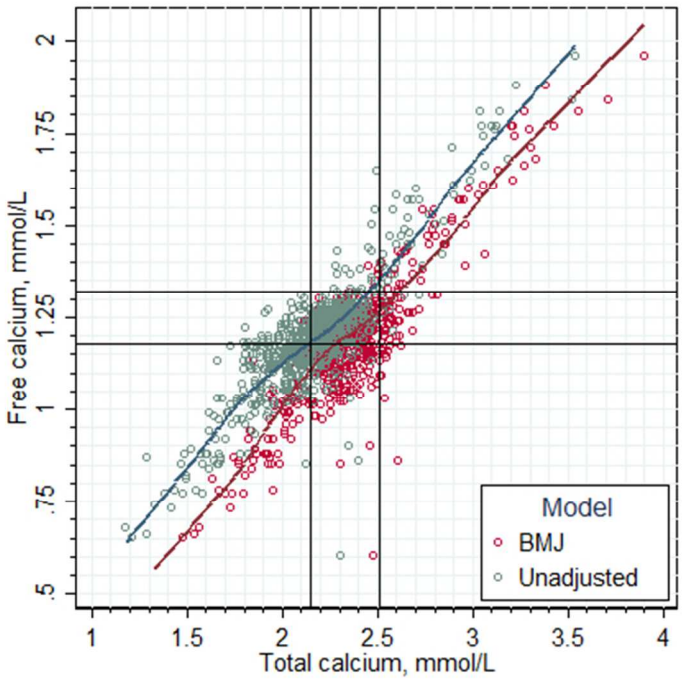
Figure 1. BMJ- and unadjusted total calcium in patients with low albumin concentrations



BMJ [6]- and unadjusted total calcium plotted against free calcium in patients with hypoalbuminemia. The reference ranges for hypo- and normocalcemia are indicated by the vertical and horisontal lines. Two locally weighted regression lines (lowess) were drawn to indicate the mean relationship between free and BMJ- and unadjusted total calcium concentrations.

References

1. Aishah AB, Foo YN. A retrospective study of serum calcium levels in a hospital population in Malaysia. Med J Malaysia. 1995;50:246-9.
2. Hastbacka J, Pettila V. Prevalence and predictive value of ionized hypocalcemia among critically ill patients. Acta Anaesth Scand. 2003;47:1264-9.
3. Baird GS. Ionized calcium. Clin Chim Acta. 2011;412:696-701.
4. Orrell DH. Albumin as an Aid to Interpretation of Serum Calcium. Clin Chim Acta. 1971;35:483-&.
5. Payne RB, Little AJ, Williams RB, Milner JR. Interpretation of Serum-Calcium in Patients with Abnormal Serum-Proteins. Br Med J. 1973;4:643-6.
6. Correcting the calcium. Br Med J. 1977;1:598.
7. Berry EM, Gupta MM, Turner SJ, Burns RR. Variation in plasma calcium with induced changes in plasma specific gravity, total protein, and albumin. Br Med J. 1973;4:640-3.
8. Hannan FM, Thakker RV. Investigating hypocalcaemia. Br Med J. 2013;346:f2213.
9. Ladenson JH, Lewis JW, Boyd JC. Failure of total calcium corrected for protein, albumin, and pH to correctly assess free calcium status. J Clin Endocrinol Metab. 1978;46:986-93.
10. Clase CM, Norman GL, Beecroft ML, Churchill DN. Albumin-corrected calcium and ionized calcium in stable haemodialysis patients. Nephrol Dial Transplant. 2000;15:1841-6.
11. Labriola L, Wallemacq P, Gulbis B, Jadoul M. The impact of the assay for measuring albumin on corrected ('adjusted') calcium concentrations. Nephrol Dial Transplant. 2009;24:1834-8.
12. Rustad P, Felding P, Franzson L, Kairisto V, Lahti A, Martensson A, et al. The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. Scand J Clin Lab Invest. 2004;64:271-84.
13. Martensson A, Rustad P, Lund H, Ossowicki H. Creatininium reference intervals for corrected methods. Scand J Clin Lab Invest. 2004;64:439-41.
14. Ceriotti F, Boyd JC, Klein G, Henny J, Queralto J, Kairisto V, et al. Reference intervals for serum creatinine concentrations: assessment of available data for global application. Clin Chem. 2008;54:559-66.
15. Jain A, Bhayana S, Vlasschaert M, House A. A formula to predict corrected calcium in haemodialysis patients. Nephrol Dial Transplant. 2008;23:2884-8.
16. Thode J, Juul-Jorgensen B, Bhatia HM, Kjaerulf-Nielsen M, Bartels PD, Fogh-Andersen N, et al. Comparison of serum total calcium, albumin-corrected total calcium, and ionized calcium in 1213 patients with suspected calcium disorders. Scand J Clin Lab Invest. 1989;49:217-23.
17. Newson RB. Interpretation of Somers' D under four simple models 2014 [Available from: http://www.imperial.ac.uk/nhli/r.newson/miscdocs/intsomd1.pdf. [accessed april 2017]
18. Gauci C, Moranne O, Fouqueray B, de la Faille R, Maruani G, Haymann JP, et al. Pitfalls of measuring total blood calcium in patients with CKD. J Am Soc Nephrol. 2008;19:1592-8.
19. Morton AR, Garland JS, Holden RM. Is the calcium correct? Measuring serum calcium in dialysis patients. Semin Dial. 2010;23:283-9.
20. Smellie WS, Association for Clinical Biochemistry's Clinical Practice S. Time to harmonise common laboratory test profiles. Br Med J. 2012;344:e1169.
21. National Kidney F. K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. Am J Kidney Dis. 2003;42:S1-201.
22. Moe S, Drueke T, Cunningham J, Goodman W, Martin K, Olgaard K, et al. Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int. 2006;69:1945-53.



215x156mm (72 x 72 DPI)

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4,5
	4	Study objectives and hypotheses	5
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6
Participants	**6**	Eligibility criteria	6
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	6
	8	Where and when potentially eligible participants were identified (setting, location and dates)	6
	9	Whether participants formed a consecutive, random or convenience series	6
Test methods	**10a**	Index test, in sufficient detail to allow replication	6,7
	10b	Reference standard, in sufficient detail to allow replication	6
	11	Rationale for choosing the reference standard (if alternatives exist)	NA
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	Reference range, p 6
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	Reference range, p 6
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	NA
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	NA
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	7
	15	How indeterminate index test or reference standard results were handled	NA
	16	How missing data on the index test and reference standard were handled	NA
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	NA
	18	Intended sample size and how it was determined	NA
RESULTS			
Participants	**19**	Flow of participants, using a diagram	NA
	20	Baseline demographic and clinical characteristics of participants	8, 15
	21a	Distribution of severity of disease in those with the target condition	NA
	21b	Distribution of alternative diagnoses in those without the target condition	NA
	22	Time interval and any clinical interventions between index test and reference standard	NA
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	NA
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	16, 17
	25	Any adverse events from performing the index test or the reference standard	NA
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	10, 11
	27	Implications for practice, including the intended use and clinical role of the index test	10, 11, 12
OTHER INFORMATION			
	28	Registration number and name of registry	NA
	29	Where the full study protocol can be accessed	NA
	30	Sources of funding and other support; role of funders	13

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

BMJ Open

Should total calcium be adjusted for albumin? – a retrospective observational study of laboratory data from central Norway

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017703.R1
Article Type:	Research
Date Submitted by the Author:	14-Sep-2017
Complete List of Authors:	Lian, Ingrid; St. Olavs hospital, Clinical Chemistry Åsberg , Arne; Sankt Olavs Hospital Universitetssykehuset i Trondheim, Department of Clinical Chemistry
Primary Subject Heading:	Diabetes and endocrinology
Secondary Subject Heading:	General practice / Family practice, Diagnostics
Keywords:	Calcium & bone < DIABETES & ENDOCRINOLOGY, albumin, calcium

Should total calcium be adjusted for albumin? – a retrospective observational study of laboratory data from central Norway

Ingrid Alsos Lian and Arne Åsberg

Department of Clinical Chemistry, St. Olavs hospital, 7006 Trondheim, Norway Ingrid Alsos Lian Resident in Clinical Chemistry Department of Clinical Chemistry, St. Olavs hospital, 7006 Trondheim, Norway Arne Åsberg Consultant in Clinical Chemistry

Correspondence to: ingrid.alsos.lian@gmail.com

Word count: 296/2917 | Tables: 3 | Figures: 1 | References: 26

Abstract

Objectives: Albumin-adjusted total calcium is often used as a surrogate marker for free calcium to evaluate hypo- or hypercalcemia. Many adjustment-formulas based on simple linear regression models have been published, and continue to be used in spite of questionable diagnostic accuracy. In the hope of finding a more pure albumin effect on total calcium, we used multiple linear regression models to adjust for other relevant variables. The regression coefficients of albumin were used to construct local adjustment formulas, and we tested whether the diagnostic accuracy was improved compared to previously published formulas and unadjusted calcium.

Design: A retrospective hospital laboratory data study.

Data sources: The local hospital laboratory data system.

Setting: Norway, 2006-2015.

Participants: 6549 patients above 2 years of age, where free calcium standardised at pH 7.40, total calcium, creatinine, albumin and phosphate had been analysed in a single blood draw, including hospitalised patients and patients from outpatient clinics and general practice**.**

Main outcome measures: Diagnostic accuracy by Harrell's c and ROC curve analysis, using free calcium standardised at pH 7.40 as a gold standard, in subgroups with eGFR ≥60 or <60 mL/minute/1.73m^2.

Results: In the subgroup with eGFR <60 mL/minute/1.73m^2, the Harrell's c of unadjusted total calcium (0.801) was significantly larger than those of the local formulas (0.790, p = 0.002) and the best formula taken from literature (0.791, p = 0.004). In the subgroup with eGFR ≥60 mL/minute/1.73m^2, no significant differences were found between these three formulas.

Conclusions: Our study shows that the diagnostic accuracy of *unadjusted* total calcium is

superior to several commonly used adjustment-formulas, and we suggest that the use of such formulas should be abandoned in clinical practice. If the clinician does not trust total calcium to reflect the calcium status of the patient, free calcium should be measured.

Strengths and limitations of this study

- Albumin-adjusted total calcium is often used as a surrogate marker for free calcium, to evaluate hypo- or hypercalcemia. Many adjustment-formulas have been published, and continue to be used in spite of questionable diagnostic accuracy in various patient populations.
- The diagnostic accuracy was evaluated using free calcium as the gold standard, both as a dichotomous one (with ROC curve analysis) and as a continuous gold standard (with Harrell's c index), the latter providing less loss of information, but corresponding to AUC when the gold standard is binary.
- This study includes a large group of both hospitalised and ambulant patients from a large regional hospital, representative of a broad spectrum of disease.
- No diagnostic information of the population was available, and a limited number of variables were included, as we wanted to retain a large sample size.

Introduction

Disturbances in calcium homeostasis are not uncommon in hospitalised patients,[1 2] although the exact prevalence in the general population is unknown. All calcium atoms in the body are ionized. In plasma, only 50% of the calcium ions are free to exert biological effects, whereas the rest are bound to proteins, mostly albumin, and a few percent are bound in complexes with anions like lactate and citrate.[3] The concentration of free calcium ions (hereafter named "free calcium") is closely regulated, and patients with abnormal albumin concentrations may have a normal concentration of free calcium despite abnormal concentration of total calcium. Unfortunately, free calcium is not as easily measured as total calcium, the latter being a part of routine test panels of large automatic clinical chemistry instruments. Accordingly, clinicians often try to compensate for an abnormal concentration of albumin, by calculating an albumin-adjusted calcium value, i.e. the clinician asks *"What would be this patient's concentration of total calcium if the albumin concentration were normal?"* Changes in the concentration of free calcium due to acidemia or alkalemia are disregarded in these cases. Several adjustment-formulas have been used,[4-7] and continue to be so,[8] in spite of their rather questionable diagnostic accuracy,[9] which may be worse than that of unadjusted calcium in certain populations.[10]

It is not completely clear why these adjustment-formulas perform so poorly. Some speculate that a certain formula is only valid for specific patient populations,[10] others that a certain formula may only be valid for certain analytical methods.[11] We hypothesise a more fundamental flaw – that the adjustment-formulas are based on wrongly formulated regression models. These formulas are estimated from patient populations with a range of total calcium and albumin concentrations, where the investigators have regressed the concentration of total

calcium against albumin, using simple linear regression.[4 5] The regression coefficient of albumin, usually in the range of 0.018-0.025,[6] then shows how much the total concentration of calcium is expected to change for one unit change in albumin concentration, comparing two hypothetical patients with different albumin concentrations. However, when making an albumin-adjustment we should use a coefficient that shows how much the total concentration of calcium is expected to change for one unit change in albumin concentration, when the patient's condition is *otherwise unchanged, specifically when the concentration of free calcium is unchanged*. To estimate that coefficient we have to regress the concentration of total calcium against albumin and free calcium, sex, age or whatever explanatory variable is relevant, not only albumin. Then the interpretation of the albumin coefficient gets in line with its use.

The purpose of this study was (i) to estimate regression coefficients for albumin from regression models which include the concentration of free calcium and other relevant explanatory variables, and (ii) to test whether the regression coefficients from these models yielded albumin-adjusted calcium values of better diagnostic accuracy than that of published formulas and unadjusted calcium.

Material and methods

Material

Data from our laboratory database were collected retrospectively, from January 1st 2006 to September 18th 2015 from all available patient records where the analysis of total calcium, free calcium standardised at pH 7.40, creatinine, albumin and phosphate had been performed in samples from the same blood draw (6567 patients). Only a single dataset (the oldest), from each patient was included. This included samples from both hospitalised patients and patients from outpatient clinics and general practice. No clinical information was collected. The population only included a very few critically ill patients, as free calcium in those patients were monitored using blood gas instruments in the intensive care units and the analytical results were not transferred to the laboratory information system. All samples were analysed at our laboratory at St. Olavs hospital, Trondheim, Norway, a full service acute care hospital.

Sample handling for analysis of free calcium

Almost all samples, from both hospitalised and ambulatory patients, consisted of venous blood drawn anaerobically into serum gel tubes with minimal use of stasis and muscle contraction, centrifuged with stopper in place within 1 hour and analysed within 24 hours after blood draw. Rarely, some samples from hospitalised patients or ambulatory patients may have been obtained anaerobically using blood gas syringes with electrolyte-balanced heparin. In these cases, the samples were analysed within 30 minutes after blood draw. Only samples with pH within 7.20-7.60 were accepted for analysis.

Laboratory analyses

Albumin, total calcium, creatinine and phosphate were assayed by colorimetric methods on

fully automated Modular P800 or Roche Cobas 6000 c501 instruments (Roche Diagnostics, Mannheim, Germany). The bromcreosol green (BCG) method was used for albumin. The creatinine assay was an enzymatic method calibrated against an isotope dilution mass spectrometry (IDMS) reference method. The concentration of free calcium was measured by an ionselective electrode mounted in an automated blood gas analyser (ABL 725 or ABL 825, Radiometer, Copenhagen, Denmark), and standardised at pH 7.40. Standard internal and external quality control procedures were followed for all analytical methods.

Reference ranges

Reference ranges for total calcium is 2.15-2.51 mmol/L,[12] 1.18-1.32 mmol/L for free calcium,[13] whereas our laboratory use age- and sex specific reference ranges for albumin and creatinine.[12 14 15]

Patient involvement

There was no direct patient involvement in the development, design or conduct of the study.

Statistical analysis

The dataset was divided into subgroups with estimated glomerular filtration rate (eGFR) below or above 60 mL/minute/1.73 m^2, as others have found different albumin coefficients in individuals with renal failure compared to individuals with normal renal function.[16] We used the full age spectrum (FAS) equation to calculate eGFR,[17] because the FAS equation is valid for both children (above 2 years of age) and adults. Values of eGFR above 200 mL/minute/1.73 m^2 were truncated at that level.[18] In addition, for patients with eGFR $\geq$ 60 mL/minute/1.73 m^2, we divided the dataset according to albumin concentrations below or above 30 g/L, as locally weighted scatterplot smoothing of total calcium against albumin

indicated nonlinearity overall, but linearity below and above 30 g/L. No such non-linear trend was observed for patients with eGFR < 60 mL/minute/1.73 m^2.

This procedure resulted in three subgroups. For each subgroup we created albumin-adjustment formulas: Adjusted calcium = calcium + coefficient × (40 - albumin), where the subgroup-specific albumin coefficients were estimated using multiple linear regression models with total calcium as the dependent variable and free calcium, albumin, phosphate, eGFR, gender, age and hospitalisation (or not) as the explanatory variables. We used backwards elimination until all remaining explanatory variables were statistically significant ($p < 0.05$). We also used simple linear regression with total calcium as the dependent variable and albumin as the sole explanatory variable, to estimate unadjusted albumin coefficients.

The diagnostic accuracy of the albumin-adjusted calcium coefficient calculated from the local formulas was compared to that of unadjusted total calcium and six other adjustment-formulas, taken from literature.[4-7 19 20] First, we used free calcium as a *dichotomous* gold standard to compare the diagnostic accuracies with receiver operating characteristic (ROC) curve analysis, where the patients were classified as hypocalcemic or not, and hypercalcemic or not, according to four different definitions of the diagnoses (cut-points for hypocalcemia: 1.12, 1.14, 1.16 and 1.18 mmol/L, and for hypercalcemia: 1.26, 1.29, 1.30 and 1.32 mmol/L). Second, we used free calcium as a *continuous* gold standard with Harrell's c index as a measure of diagnostic accuracy. This index is related to the area under the ROC curve. Both measures are 0.5 at no diagnostic accuracy and 1.0 at perfect diagnostic accuracy. Harrell's c takes on the same value as the area under the ROC curve when the gold standard is binary.[21] The diagnostic accuracy was studied for subgroups with eGFR below or above 60 mL/minute/1.73 m^2.

Laboratory data was extracted using SAS (version 9.2 for Windows, SAS Institute, NC, USA) and analysed using STATA (version 14.1 for Windows, StataCorp LP, TX, USA). Harrell's c index was calculated by the "somersd" procedure and differences between two indexes by the "lincom" procedure. Differences between proportions were tested by the chi-square test and differences between medians by the Mann Whitney U test. $P < 0.05$ was considered statistically significant.

Results

Clinical data

Data from a total of 6567 patients were collected, from 3895 women (59.3%) and 2672 men (40.7%). We excluded 18 patients below 2 years of age, as the FAS eGFR equation is validated for individuals aged 2 years or older.[17] Characteristics of the 6549 included patients are given in table 1. The hospitalised patients differed significantly from the out-patients in all characteristics.

Albumin coefficients

The results of simple and multiple linear regression analyses are given in table 2. The unadjusted regression coefficients of albumin were significantly different below and above 30 g/L for patients with eGFR ≥ 60 mL/minute/1.73 m^2 ($p < 0.001$). With multiple linear regression, patients with eGFR ≥ 60 mL/minute/1.73 m^2 and albumin < 30 g/L had a 32.5% higher adjusted regression coefficient than patients with eGFR ≥ 60 mL/minute/1.73 m^2 and albumin ≥ 30 g/L.

Diagnostic accuracy

The diagnostic accuracy of unadjusted calcium and albumin-adjusted calcium values calculated from the locally constructed formulas and formulas taken from literature are shown in figure 1 (ROC curve analysis) and table 3 (Harrell's c index). In patients with eGFR < 60 ml/minutes/1.73 m^2, unadjusted calcium outperformed all albumin-adjustment formulas in diagnosing hypocalcemia, independent of the definitions of hypocalcemia used in this study ($p < 0.001$ at all definitions when compared to the formula of James et al.[20] (figure 1b)). In patients with eGFR ≥ 60 ml/minutes/1.73 m^2, unadjusted calcium was not inferior to any

albumin-adjustment formulas in diagnosing hypocalcemia (figure 1a). In diagnosing hypercalcemia, unadjusted calcium performed somewhat worse than some albumin-adjustment formulas (figure 1c and 1d). When free calcium was treated as a continuous gold standard, using Harrell's c index, unadjusted calcium performed significantly better than the best calcium-adjustment formula (the formula of James et al.,[20] $p = 0.004$) and the locally constructed formulas ($p = 0.002$) in patients with eGFR < 60 ml/minutes/1.73 m^2. In patients with eGFR ≥ 60 ml/minutes/1.73 m^2 unadjusted calcium was not inferior to those formulas and ($p = 0.43$ versus the James et al. formula and $p = 0.97$ versus the locally constructed formulas) and significantly better than the other formulas ($p < 0.001$ in all cases).

Discussion

In this work, we estimated regression coefficients for albumin that reflected how much total calcium changes per unit change in albumin, adjusted for other relevant variables. To our knowledge, that has not been done before. Although theoretically sound and lower albumin coefficients were found (table 2), this procedure was nevertheless a disappointment, as the locally constructed formulas performed worse than unadjusted calcium in the subgroup of patients with eGFR < 60 mL/minutes/1.73 m^2, and no better than unadjusted calcium in the subgroup with eGFR ≥ 60 mL/minutes/1.73 m^2 (table 3). This was even more disappointing, as the local formulas were derived from and tested on the same dataset, so one would expect that their performance were positively biased. In fact, our locally constructed formulas performed very much like the formula of James et al.[20] Given that our regression coefficients for most patients are the same as or close to the value of 0.012 in the James et al. formula, equal performance is no surprise. It is more remarkable that we estimated about the same regression coefficients as James et al. when the populations, albumin methods and regression methods were different.[20] However, James et al. did not adjust for other relevant variables, so only the unadjusted albumin coefficients can be directly compared. Those coefficients were higher in our population than in the population of James et al. (table 2), probably due to different albumin methods (bromcresol green versus bromcresol purple) as well different populations.

The diagnostic accuracy of albumin-adjustment formulas has been questioned previously. Almost 40 years ago, Ladenson et al.[9] compared 13 different adjustment formulas in a population of 375 hospitalised patients and 53 controls, among them the albumin-adjustment formulas of Orrell,[4] Berry[7] and Payne[5], and found that none correlated better with free calcium than unadjusted calcium. We have found no evidence in the literature supporting that

albumin-adjusted calcium is superior to unadjusted calcium in this aspect. Our study includes a relatively large group of both hospitalised and ambulant patients from a large regional hospital, representing an unselected population with a broad spectrum of disease. Compared to Ladenson et al.,[9] our findings are strengthened by a much larger study population. In addition, we have evaluated the diagnostic accuracy using free calcium both as a continuous gold standard (with Harrell's c index) and as a dichotomous one (with ROC curve analysis).

The various adjustment-formulas use different normal values of albumin. We normalised to 40 g/L, as did Payne,[5] while Orrell[4] used 34 g/L and Berry[7] 46 g/L. The choice of normal albumin value does not influence the diagnostic accuracy, because adjusted calcium = calcium + coefficient × (normal albumin - albumin) = calcium + coefficient × normal albumin - coefficient × albumin = calcium + constant + coefficient × albumin. Adding a constant to the value of a diagnostic marker does not change its diagnostic accuracy. The choice of normal albumin value does, however, influence the optimal cut-off value of albumin-adjusted calcium. Finding the optimal cut-off value could be done by ROC analysis if the prevalence of the clinical condition and the consequences of false and true positive and negative results are known,[22] but such an analysis was beyond the scope of this work.

As judged by ROC curve analysis, some of the other formulas taken from literature performed rather poorly in the diagnosis of hypocalcemia in all patients (figure 1a and 1b), and in the diagnosis of hypercalcemia in patients with eGFR < 60 mL/minute/1.73 m^2 (figure 1d). In the diagnosis of hypercalcemia in patients with eGFR ≥ 60 mL/minute/1.73 m^2, they all performed rather well (figure 1c). As judged by the Harrell's c index, unadjusted calcium was the most accurate diagnostic test in patients with eGFR < 60 mL/minute/1.73 m^2, and not inferior to any formula in patients with eGFR ≥ 60 mL/minute/1.73 m^2. The commonly used

BMJ-formula ($Calcium_{adj}$ (mmol/L) = total calcium (mmol/L) + 0.02 × (40 - albumin (mmol/L), suggested in BMJ in 1977[6]), was significantly less accurate than unadjusted calcium in both eGFR groups. All calcium measures performed better in patients with eGFR < 60 mL/minute/1.73 m^2 than in those with eGFR ≥ 60 mL/minute/1.73 m^2. The ROC curve analyses partly corroborated this; however, in the diagnosis of hypercalcemia in patients with eGFR ≥ 60 mL/minute/1.73 m^2, some albumin-adjustment formulas performed slightly better than unadjusted calcium. Furthermore, the calcium measures were no better in patients with eGFR < 60 mL/minute/1.73 m^2 than in patients with eGFR ≥ 60 mL/minute/1.73 m^2. We have no explanation of this divergence between the two methods of evaluating diagnostic accuracy, other than the information loss from dichotomisation of the continuous gold standard in the ROC curve analyses. This loss of information was partly taken into consideration, as we used four different definitions of hypo- and hypercalcemia in the ROC curves analyses. We extended both definitions downwards from our reference limits of 1.18 and 1.32 mmol/L, as more recent work indicates that our reference limits may be somewhat high.[23] As expected, the area under the ROC curves increased when hypo- and hypercalcemia were defined more stringently, i.e. when the diagnoses represented more pathological cases.

Limitations of the study

Some limitations of our study should be mentioned. First, the use of pH-adjusted free calcium as a gold standard could be questioned. Although the actual concentration of free calcium in correctly sampled blood specimens should be the most relevant measure of calcium status, Thode at al. found that pH-adjusted free calcium was as useful as the actual (unadjusted) free calcium in 183 patients with various calcium disorders.[24] Anyway, pH-adjusted free calcium was the only measure we could use, as the actual (unadjusted) free calcium was recorded in only 26 patients. Second, as no diagnostic information was available to us we do not know

whether our findings are applicable to every clinical condition. However, the renal function could be estimated. The fraction of patients with an eGFR less than 60 mL/minute/1.73 m^2 was very different in ambulant and hospitalised patients (18 % versus 62 %), indicating that reduced renal function was more prevalent in hospitalised patients and/or that free calcium was more likely to be requested in hospitalised patients with reduced renal function. The relatively large number of patients with reduced renal function in the study population may be an advantage, as we know from this and other studies[16,25 26] that albumin-adjustment formulas perform differently in patients with and without renal failure. Third, we did not collect data on sodium, magnesium and parathyroid hormone. Such data could have been included for a better estimate of the albumin coefficient. However, inclusion of more variables in the same blood draw would significantly have reduced the sample size. We wanted to keep the samples size as large as possible to get a reliable estimate of the albumin coefficient.

Conclusion

We found that the diagnostic accuracy of unadjusted calcium in general is superior to that of albumin-adjusted total calcium based on formulas from literature, and even to that of locally constructed adjustment-formulas especially adapted to our dataset. Despite that many have questioned the diagnostic accuracy of albumin-adjustment formulas previously, they continue to be used in general clinical practice. We believe that the clinician should order measurement of free calcium instead of albumin-adjusted calcium in patients where total calcium is not to be trusted, as the analysis of free calcium is now widely available.

No competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the

submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical consideration

The study was carried out in full accordance with the ethical principles of the Declaration of Helsinki. According to the Norwegian Health Research Act, this type of project which uses anonymous information from local health registers, is not required to be evaluated by the Norwegian Regional Ethical Committee (REC).

Research funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Acknowledgments

None declared.

Data sharing

The full dataset is available upon request from the corresponding author (ingrid.alsos.lian@gmail.com). Informed consent for data sharing was not obtained, but the presented data are anonymised and risk of identification is low.

Contributors

IAL and AÅ both contributed to the acquisition, analysis, and interpretation of data for the work, the drafting the work or revising it critically for important intellectual content. AÅ was the main contributor in the conception and design of the work. IAL is the guarantor.

Table 1. Characteristics of the study population. Continuous variables are given as medians (2.5-97.5 percentile).

Characteristic	Inpatients	Outpatients	p - value
Number of individuals	778	5771	
Percent women	45.1	61.2	< 0.0001
Age (years)	67 (7-90)	47 (13-82)	< 0.0001
Total calcium (mmol/L)	2.22 (1.61-3.03)	2.31 (2.09-2.61)	< 0.0001
percent hypocalcemia	35.2	5.2	< 0.0001
percent hypercalcemia	10.8	5.4	< 0.0001
Free calcium (mmol/L)	1.19 (0.85-1.67)	1.23 (1.13-1.39)	< 0.0001
percent hypocalcemia	41.7	9.2	< 0.0001
percent hypercalcemia	11.3	5.3	< 0.0001
Albumin (g/L)	33 (18-45)	41 (34-46)	< 0.0001
percent hypoalbuminemia	60.0	4.9	< 0.0001
percent hyperalbuminemia	0.6	2.0	0.0063
eGFR (ml/minute/1.73m^2)	39 (7-171)	96 (19-154)	< 0.0001
percent < 60	62.3	18.2	< 0.0001
Phosphate (mmol/L)	1.18 (0.48-2.68)	1.00 (0.64-1.54)	< 0.0001

Table 2. Results of simple linear regression of total calcium against albumin, and multiple linear regression of total calcium against albumin and other relevant variables. Only the adjusted albumin coefficients were used to construct the local group-specific formulas for albumin-adjusted calcium.

	Simple linear regression	Multiple linear regression	
Subpopulation	Unadjusted albumin coefficient (95% CI)	Significant variables	Adjusted albumin coefficient (95% CI)
All with eGFR ≥ 60 mL/minute/1.73 m^2, n=5013	0.0167 (0.0158-0.0176)	Albumin, free calcium, gender, age, eGFR, phosphate	0.0126 (0.0121-0.0132)
eGFR ≥ 60 mL/minute/1.73 m^2 and albumin < 30 g/L, n=103	0.0282 (0.0158-0.0406)	Albumin, free calcium	0.0159 (0.0112-0.0206)
eGFR ≥ 60 mL/minute/1.73 m^2 and albumin ≥ 30 g/L, n=4910	0.0154 (0.0142-0.0166)	Albumin, free calcium, gender, age, eGFR, phosphate, hospitalisation status	0.0120 (0.0113-0.0128)
All with eGFR < 60 mL/minute/1.73 m^2, n=1536	0.0160 (0.0140-0.0181)	Albumin, free calcium, age, eGFR, phosphate	0.0123 (0.0113-0.0133)

Table 3. Agreement between total calcium and free calcium as measured by Harrell's c index (95% CI) in patients with eGFR above or below normal 60 mL/minute/1.73 m^2.

Adjustment formula	eGFR ≥ 60 mL/minute/1.73 m^2 (n=5013)	eGFR < 60 mL/minute/1.73 m^2 (n=1536)
None	0.749 (0.741-0.758)	0.801 (0.788-0.813)
Local*	0.749 (0.741-0.758)	0.790 (0.776-0.803)
James[20]	0.751 (0.743-0.759)	0.791 (0.777-0.804)
Orrell[4]	0.736 (0.728-0.745)	0.766 (0.751-0.781)
BMJ[6]	0.728 (0.719-0.737)	0.753 (0.738-0.769)
Thode[16]	0.726 (0.717-0.735)	0.747 (0.731-0.763)
Berry[7]	0.716 (0.707-0.725)	0.736 (0.720-0.752)
Payne[5]	0.707 (0.698-0.716)	0.723 (0.707-0.740)

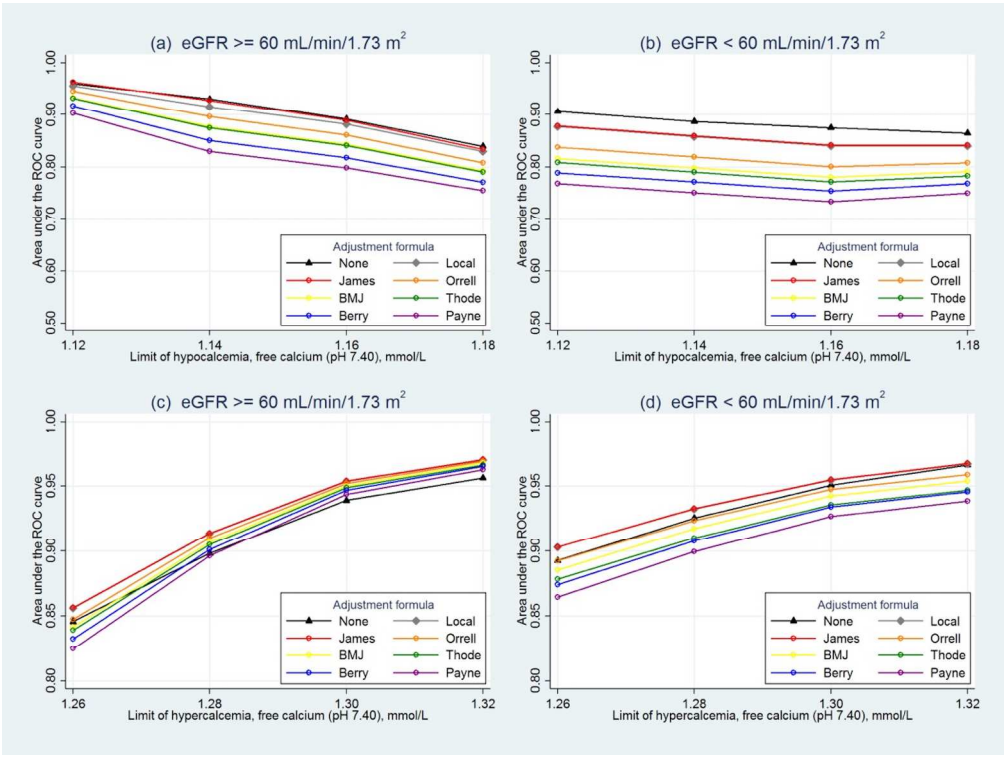
*The local adjustment-formulas were constructed using three subgroup-specific albumin coefficients, see Methods

Figure legend

Figure 1. Accuracy in the diagnosis of hypocalcemia (a and b) and hypercalcemia (c and d) in patients with eGFR above (a and c) or below (b and d) 60 mL/minute/1.73 m^2, given as the area under the ROC curve for various albumin-adjustment formulas and for unadjusted calcium.

References

1. Aishah AB, Foo YN. A retrospective study of serum calcium levels in a hospital population in Malaysia. *Med J Malaysia* 1995;50(3):246-9.
2. Hastbacka J, Pettila V. Prevalence and predictive value of ionized hypocalcemia among critically ill patients. *Acta Anaesth Scand* 2003;47(10):1264-69.
3. Baird GS. Ionized calcium. *Clin Chim Acta* 2011;412(9-10):696-701.
4. Orrell DH. Albumin as an Aid to Interpretation of Serum Calcium. *Clin Chim Acta* 1971;35(2):483-9.
5. Payne RB, Little AJ, Williams RB, et al. Interpretation of Serum-Calcium in Patients with Abnormal Serum-Proteins. *BMJ* 1973;4(5893):643-46.
6. Correcting the calcium. *BMJ* 1977;1(6061):598.
7. Berry EM, Gupta MM, Turner SJ, et al. Variation in plasma calcium with induced changes in plasma specific gravity, total protein, and albumin. *BMJ* 1973;4(5893):640-3.
8. Hannan FM, Thakker RV. Investigating hypocalcaemia. *BMJ* 2013;346:f2213.
9. Ladenson JH, Lewis JW, Boyd JC. Failure of total calcium corrected for protein, albumin, and pH to correctly assess free calcium status. *J Clin Endocrinol Metab* 1978;46(6):986-93.
10. Clase CM, Norman GL, Beecroft ML, et al. Albumin-corrected calcium and ionized calcium in stable haemodialysis patients. *Nephrol Dial Transplant* 2000;15(11):1841-6.
11. Labriola L, Wallemacq P, Gulbis B, et al. The impact of the assay for measuring albumin on corrected ('adjusted') calcium concentrations. *Nephrol Dial Transplant* 2009;24(6):1834-8.
12. Rustad P, Felding P, Franzson L, et al. The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. *Scand J Clin Lab Invest* 2004;64(4):271-84.
13. Olesen H. Kompendium i Laboratoriemedicin. Copenhagen: Amtrådsforeningen i Danmark, 1988.
14. Martensson A, Rustad P, Lund H, et al. Creatininium reference intervals for corrected methods. *Scand J Clin Lab Invest* 2004;64(4):439-41.
15. Ceriotti F, Boyd JC, Klein G, et al. Reference intervals for serum creatinine concentrations: assessment of available data for global application. *Clin Chem* 2008;54(3):559-66.
16. Jain A, Bhayana S, Vlasschaert M, et al. A formula to predict corrected calcium in haemodialysis patients. *Nephrol Dial Transplant* 2008;23(9):2884-8.
17. Pottel H, Hoste L, Dubourg L, et al. An estimated glomerular filtration rate equation for the full age spectrum. *Nephrol Dial Transplant* 2016;31(5):798-806.
18. Coresh J, Selvin E, Stevens LA, et al. Prevalence of chronic kidney disease in the United States. *JAMA* 2007;298(17):2038-47.
19. Thode J, Juul-Jorgensen B, Bhatia HM, et al. Comparison of serum total calcium, albumin-corrected total calcium, and ionized calcium in 1213 patients with suspected calcium disorders. *Scand J Clin Lab Invest* 1989;49(3):217-23.
20. James MT, Zhang J, Lyon AW, et al. Derivation and internal validation of an equation for albumin-adjusted calcium. *BMC Clin Pathol* 2008;8:12.
21. Newson RB. Interpretation of Somers' D under four simple models [Edited 2014] Available from: http://www.imperial.ac.uk/nhli/r.newson/miscdocs/intsomd1.pdf.
22. Metz CE. Basic principles of ROC analysis. *Semin Nucl Med* 1978;8(4):283-98.
23. Klaestrup E, Trydal T, Pedersen JF, et al. Reference intervals and age and gender dependency for arterial blood gases and electrolytes in adults. *Clin Chem Lab Med* 2011;49(9):1495-500.
24. Thode J, Holmegaard SN, Transbol I, et al. Adjusted ionized calcium (at pH 7.4) and actual ionized calcium (at actual pH) in capillary blood compared for clinical evaluation of patients with disorders of calcium metabolism. *Clin Chem* 1990;36(3):541-4.
25. Gauci C, Moranne O, Fouqueray B, et al. Pitfalls of measuring total blood calcium in patients with CKD. *J Am Soc Nephrol* 2008;19(8):1592-8.
26. Morton AR, Garland JS, Holden RM. Is the calcium correct? Measuring serum calcium in dialysis patients. *Semin Dial* 2010;23(3):283-9.



1960x1470mm (72 x 72 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2+3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Introduction, p5+6
Objectives	3	State specific objectives, including any pre-specified hypotheses	Introduction, p6
Methods			
Study design	4	Present key elements of study design early in the paper	Abstract p2+3, introduction p6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Mat and met p7+8
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Mat and met p7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Mat and met p7-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Mat and met p7-8
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	Mat and met p7, Res p11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Statistical analyses p8-10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Statistical analyses p8-10
		(*b*) Describe any methods used to examine subgroups and interactions	

		(*c*) Explain how missing data were addressed	NA, no missing data
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Mat and met p7, Res p11
		(b) Give reasons for non-participation at each stage	Res p11
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Material p6, Results p11, table 1 p19
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	Results p11-12, tables and figures p19-22
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Results p11-12, tables p19-21
		(*b*) Report category boundaries when continuous variables were categorized	Mat and met P8+9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	Discussion p13, conclusion p16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Discussion p15-16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Discussion p13-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	Discussion p14-16
Other information			

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Should total calcium be adjusted for albumin? – a retrospective observational study of laboratory data from central Norway

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017703.R2
Article Type:	Research
Date Submitted by the Author:	25-Nov-2017
Complete List of Authors:	Lian, Ingrid; St. Olavs hospital, Clinical Chemistry Åsberg , Arne; Sankt Olavs Hospital Universitetssykehuset i Trondheim, Department of Clinical Chemistry
Primary Subject Heading:	Diabetes and endocrinology
Secondary Subject Heading:	General practice / Family practice, Diagnostics
Keywords:	Calcium & bone < DIABETES & ENDOCRINOLOGY, albumin, calcium

Should total calcium be adjusted for albumin? – a retrospective observational study of laboratory data from central Norway

Ingrid Alsos Lian and Arne Åsberg

Department of Clinical Chemistry, St. Olavs hospital, 7006 Trondheim, Norway Ingrid Alsos Lian Resident in Clinical Chemistry Department of Clinical Chemistry, St. Olavs hospital, 7006 Trondheim, Norway Arne Åsberg Consultant in Clinical Chemistry

Correspondence to: ingrid.alsos.lian@gmail.com

Word count: 296/2917 | Tables: 3 | Figures: 1 | References: 26

Abstract

Objectives: Albumin-adjusted total calcium is often used as a surrogate marker for free calcium to evaluate hypo- or hypercalcemia. Many adjustment-formulas based on simple linear regression models have been published, and continue to be used in spite of questionable diagnostic accuracy. In the hope of finding a more pure albumin effect on total calcium, we used multiple linear regression models to adjust for other relevant variables. The regression coefficients of albumin were used to construct local adjustment formulas, and we tested whether the diagnostic accuracy was improved compared to previously published formulas and unadjusted calcium.

Design: A retrospective hospital laboratory data study.

Data sources: The local hospital laboratory data system.

Setting: Norway, 2006-2015.

Participants: 6549 patients above 2 years of age, where free calcium standardised at pH 7.40, total calcium, creatinine, albumin and phosphate had been analysed in a single blood draw, including hospitalised patients and patients from outpatient clinics and general practice**.**

Main outcome measures: Diagnostic accuracy by Harrell's c and ROC curve analysis, using free calcium standardised at pH 7.40 as a gold standard, in subgroups with eGFR ≥60 or <60 mL/minute/1.73m^2.

Results: In the subgroup with eGFR <60 mL/minute/1.73m^2, the Harrell's c of unadjusted total calcium (0.801) was significantly larger than those of the local formulas (0.790, p = 0.002) and the best formula taken from literature (0.791, p = 0.004). In the subgroup with eGFR ≥60 mL/minute/1.73m^2, no significant differences were found between these three formulas.

Conclusions: Our study shows that the diagnostic accuracy of *unadjusted* total calcium is

superior to several commonly used adjustment-formulas, and we suggest that the use of such formulas should be abandoned in clinical practice. If the clinician does not trust total calcium to reflect the calcium status of the patient, free calcium should be measured.

Strengths and limitations of this study

- Albumin-adjusted total calcium is often used as a surrogate marker for free calcium, to evaluate hypo- or hypercalcemia. Many adjustment-formulas have been published, and continue to be used in spite of questionable diagnostic accuracy in various patient populations.
- The diagnostic accuracy was evaluated using free calcium as the gold standard, both as a dichotomous one (with ROC curve analysis) and as a continuous gold standard (with Harrell's c index), the latter providing less loss of information, but corresponding to AUC when the gold standard is binary.
- This study includes a large group of both hospitalised and ambulant patients from a large regional hospital, representative of a broad spectrum of disease.
- No diagnostic information of the population was available, and a limited number of variables were included, as we wanted to retain a large sample size.

Introduction

Disturbances in calcium homeostasis are not uncommon in hospitalised patients,[1 2] although the exact prevalence in the general population is unknown. All calcium atoms in the body are ionized. In plasma, only 50% of the calcium ions are free to exert biological effects, whereas the rest are bound to proteins, mostly albumin, and a few percent are bound in complexes with anions like lactate and citrate.[3] The concentration of free calcium ions (hereafter named "free calcium") is closely regulated, and patients with abnormal albumin concentrations may have a normal concentration of free calcium despite abnormal concentration of total calcium. Unfortunately, free calcium is not as easily measured as total calcium, the latter being a part of routine test panels of large automatic clinical chemistry instruments. Accordingly, clinicians often try to compensate for an abnormal concentration of albumin, by calculating an albumin-adjusted calcium value, i.e. the clinician asks *"What would be this patient's concentration of total calcium if the albumin concentration were normal?"* Changes in the concentration of free calcium due to acidemia or alkalemia are disregarded in these cases. Several adjustment-formulas have been used,[4-7] and continue to be so,[8] in spite of their rather questionable diagnostic accuracy,[9] which may be worse than that of unadjusted calcium in certain populations.[10]

It is not completely clear why these adjustment-formulas perform so poorly. Some speculate that a certain formula is only valid for specific patient populations,[10] others that a certain formula may only be valid for certain analytical methods.[11] We hypothesise a more fundamental flaw – that the adjustment-formulas are based on wrongly formulated regression models. These formulas are estimated from patient populations with a range of total calcium and albumin concentrations, where the investigators have regressed the concentration of total

calcium against albumin, using simple linear regression.[4 5] The regression coefficient of albumin, usually in the range of 0.018-0.025,[6] then shows how much the total concentration of calcium is expected to change for one unit change in albumin concentration, comparing two hypothetical patients with different albumin concentrations. However, when making an albumin-adjustment we should use a coefficient that shows how much the total concentration of calcium is expected to change for one unit change in albumin concentration, when the patient's condition is *otherwise unchanged, specifically when the concentration of free calcium is unchanged*. To estimate that coefficient we have to regress the concentration of total calcium against albumin and free calcium, sex, age or whatever explanatory variable is relevant, not only albumin. Then the interpretation of the albumin coefficient gets in line with its use.

The purpose of this study was (i) to estimate regression coefficients for albumin from regression models which include the concentration of free calcium and other relevant explanatory variables, and (ii) to test whether the regression coefficients from these models yielded albumin-adjusted calcium values of better diagnostic accuracy than that of published formulas and unadjusted calcium.

Material and methods

Material

Data from our laboratory database were collected retrospectively, from January 1st 2006 to September 18th 2015 from all available patient records where the analysis of total calcium, free calcium standardised at pH 7.40, creatinine, albumin and phosphate had been performed in samples from the same blood draw (6567 patients). Only a single dataset (the oldest), from each patient was included. This included samples from both hospitalised patients and patients from outpatient clinics and general practice. No clinical information was collected. The population only included a very few critically ill patients, as free calcium in those patients were monitored using blood gas instruments in the intensive care units and the analytical results were not transferred to the laboratory information system. All samples were analysed at our laboratory at St. Olavs hospital, Trondheim, Norway, a full service acute care hospital.

Sample handling for analysis of free calcium

Almost all samples, from both hospitalised and ambulatory patients, consisted of venous blood drawn anaerobically into serum gel tubes with minimal use of stasis and muscle contraction, centrifuged with stopper in place within 1 hour and analysed within 24 hours after blood draw. Rarely, some samples from hospitalised patients or ambulatory patients may have been obtained anaerobically using blood gas syringes with electrolyte-balanced heparin. In these cases, the samples were analysed within 30 minutes after blood draw. Only samples with pH within 7.20-7.60 were accepted for analysis.

Laboratory analyses

Albumin, total calcium, creatinine and phosphate were assayed by colorimetric methods on

fully automated Modular P800 or Roche Cobas 6000 c501 instruments (Roche Diagnostics, Mannheim, Germany). The bromcreosol green (BCG) method was used for albumin. The creatinine assay was an enzymatic method calibrated against an isotope dilution mass spectrometry (IDMS) reference method. The concentration of free calcium was measured by an ionselective electrode mounted in an automated blood gas analyser (ABL 725 or ABL 825, Radiometer, Copenhagen, Denmark), and standardised at pH 7.40. Standard internal and external quality control procedures were followed for all analytical methods.

Reference ranges

Reference ranges for total calcium is 2.15-2.51 mmol/L,[12] 1.18-1.32 mmol/L for free calcium,[13] whereas our laboratory use age- and sex specific reference ranges for albumin and creatinine.[12 14 15]

Patient involvement

There was no direct patient involvement in the development, design or conduct of the study.

Statistical analysis

The dataset was divided into subgroups with estimated glomerular filtration rate (eGFR) below or above 60 mL/minute/1.73 m^2, as others have found different albumin coefficients in individuals with renal failure compared to individuals with normal renal function.[16] We used the full age spectrum (FAS) equation to calculate eGFR,[17] because the FAS equation is valid for both children (above 2 years of age) and adults. Values of eGFR above 200 mL/minute/1.73 m^2 were truncated at that level.[18] In addition, for patients with eGFR $\geq$ 60 mL/minute/1.73 m^2, we divided the dataset according to albumin concentrations below or above 30 g/L, as locally weighted scatterplot smoothing of total calcium against albumin

indicated nonlinearity overall, but linearity below and above 30 g/L. No such non-linear trend was observed for patients with eGFR < 60 mL/minute/1.73 m^2.

This procedure resulted in three subgroups. For each subgroup we created albumin-adjustment formulas: Adjusted calcium = calcium + coefficient × (40 - albumin), where the subgroup-specific albumin coefficients were estimated using multiple linear regression models with total calcium as the dependent variable and free calcium, albumin, phosphate, eGFR, gender, age and hospitalisation (or not) as the explanatory variables. We used backwards elimination until all remaining explanatory variables were statistically significant ($p < 0.05$). We also used simple linear regression with total calcium as the dependent variable and albumin as the sole explanatory variable, to estimate unadjusted albumin coefficients.

The diagnostic accuracy of the albumin-adjusted calcium coefficient calculated from the local formulas was compared to that of unadjusted total calcium and six other adjustment-formulas, taken from literature.[4-7 19 20] First, we used free calcium as a *dichotomous* gold standard to compare the diagnostic accuracies with receiver operating characteristic (ROC) curve analysis, where the patients were classified as hypocalcemic or not, and hypercalcemic or not, according to four different definitions of the diagnoses (cut-points for hypocalcemia: 1.12, 1.14, 1.16 and 1.18 mmol/L, and for hypercalcemia: 1.26, 1.29, 1.30 and 1.32 mmol/L). Second, we used free calcium as a *continuous* gold standard with Harrell's c index as a measure of diagnostic accuracy. This index is related to the area under the ROC curve. Both measures are 0.5 at no diagnostic accuracy and 1.0 at perfect diagnostic accuracy. Harrell's c takes on the same value as the area under the ROC curve when the gold standard is binary.[21] The diagnostic accuracy was studied for subgroups with eGFR below or above 60 mL/minute/1.73 m^2.

Laboratory data was extracted using SAS (version 9.2 for Windows, SAS Institute, NC, USA) and analysed using STATA (version 14.1 for Windows, StataCorp LP, TX, USA). Harrell's c index was calculated by the "somersd" procedure and differences between two indexes by the "lincom" procedure. Differences between proportions were tested by the chi-square test and differences between medians by the Mann Whitney U test. $P < 0.05$ was considered statistically significant.

Results

Clinical data

Data from a total of 6567 patients were collected, from 3895 women (59.3%) and 2672 men (40.7%). We excluded 18 patients below 2 years of age, as the FAS eGFR equation is validated for individuals aged 2 years or older.[17] Characteristics of the 6549 included patients are given in table 1. The hospitalised patients differed significantly from the out-patients in all characteristics.

Albumin coefficients

The results of simple and multiple linear regression analyses are given in table 2. The unadjusted regression coefficients of albumin were significantly different below and above 30 g/L for patients with eGFR ≥ 60 mL/minute/1.73 m^2 ($p < 0.001$). With multiple linear regression, patients with eGFR ≥ 60 mL/minute/1.73 m^2 and albumin < 30 g/L had a 32.5% higher adjusted regression coefficient than patients with eGFR ≥ 60 mL/minute/1.73 m^2 and albumin ≥ 30 g/L.

Diagnostic accuracy

The diagnostic accuracy of unadjusted calcium and albumin-adjusted calcium values calculated from the locally constructed formulas and formulas taken from literature are shown in figure 1 (ROC curve analysis) and table 3 (Harrell's c index). In patients with eGFR ≥ 60 ml/minutes/1.73 m^2, unadjusted calcium was not inferior to any albumin-adjustment formulas in diagnosing hypocalcemia (figure 1a). In patients with eGFR < 60 ml/minutes/1.73 m^2, unadjusted calcium outperformed all albumin-adjustment formulas in diagnosing hypocalcemia, independent of the definitions of hypocalcemia used in this study ($p < 0.001$ at

all definitions when compared to the formula of James et al.[20] (figure 1b)). In diagnosing hypercalcemia, unadjusted calcium performed somewhat worse than some albumin-adjustment formulas (figure 1c and 1d). When free calcium was treated as a continuous gold standard, using Harrell's c index, unadjusted calcium performed significantly better than the best calcium-adjustment formula (the formula of James et al.,[20] $p = 0.004$) and the locally constructed formulas ($p = 0.002$) in patients with eGFR < 60 ml/minutes/1.73 m^2. In patients with eGFR ≥ 60 ml/minutes/1.73 m^2 unadjusted calcium was not inferior to those formulas and ($p = 0.43$ versus the James et al. formula and $p = 0.97$ versus the locally constructed formulas) and significantly better than the other formulas ($p < 0.001$ in all cases).

Discussion

In this work, we estimated regression coefficients for albumin that reflected how much total calcium changes per unit change in albumin, adjusted for other relevant variables. To our knowledge, that has not been done before. Although theoretically sound and lower albumin coefficients were found (table 2), this procedure was nevertheless a disappointment, as the locally constructed formulas performed worse than unadjusted calcium in the subgroup of patients with eGFR < 60 mL/minutes/1.73 m^2, and no better than unadjusted calcium in the subgroup with eGFR ≥ 60 mL/minutes/1.73 m^2 (table 3). This was even more disappointing, as the local formulas were derived from and tested in the same dataset, so one would expect that their performance were positively biased. In fact, our locally constructed formulas performed very much like the formula of James et al.[20] Given that our regression coefficients for most patients are the same as or close to the value of 0.012 in the James et al. formula, equal performance is no surprise. It is more remarkable that we estimated about the same regression coefficients as James et al. when the populations, albumin methods and regression methods were different.[20] However, James et al. did not adjust for other relevant variables, so only the unadjusted albumin coefficients can be directly compared. Those coefficients were higher in our population than in the population of James et al. (table 2), probably due to different albumin methods (bromcresol green versus bromcresol purple) as well different populations. Our finding of a statistically significantly higher adjusted regression coefficient in patients with albumin ≥ 30 g/L compared to those with albumin < 30 g/L in the group with eGFR ≥ 60 mL/minute/1.73 m2 (table 2) may not be clinically significant, as our formulas did not outperform the formula of James et al. who used the same coefficient in all patient groups.

The diagnostic accuracy of albumin-adjustment formulas has been questioned previously. Almost 40 years ago, Ladenson et al.[9] compared 13 different adjustment formulas in a

population of 375 hospitalised patients and 53 controls, among them the albumin-adjustment formulas of Orrell,[4] Berry[7] and Payne[5], and found that none correlated better with free calcium than unadjusted calcium. We have found no evidence in the literature supporting that albumin-adjusted calcium is superior to unadjusted calcium in this aspect. Our study includes a relatively large group of both hospitalised and ambulant patients from a large regional hospital, representing an unselected population with a broad spectrum of disease. Compared to Ladenson et al.,[9] our findings are strengthened by a much larger study population. In addition, we have evaluated the diagnostic accuracy using free calcium both as a continuous gold standard (with Harrell's c index) and as a dichotomous one (with ROC curve analysis).

The various adjustment-formulas use different normal values of albumin. We normalised to 40 g/L, as did Payne,[5] while Orrell[4] used 34 g/L and Berry[7] 46 g/L. The choice of normal albumin value does not influence the diagnostic accuracy, because adjusted calcium = calcium + coefficient × (normal albumin - albumin) = calcium + coefficient × normal albumin - coefficient × albumin = calcium + constant + coefficient × albumin. Adding a constant to the value of a diagnostic marker does not change its diagnostic accuracy. The choice of normal albumin value does, however, influence the optimal cut-off value of albumin-adjusted calcium. Finding the optimal cut-off value could be done by ROC analysis if the prevalence of the clinical condition and the consequences of false and true positive and negative results are known,[22] but such an analysis was beyond the scope of this work.

As judged by ROC curve analysis, some of the other formulas taken from literature performed rather poorly in the diagnosis of hypocalcemia in all patients (figure 1a and 1b), and in the diagnosis of hypercalcemia in patients with eGFR < 60 mL/minute/1.73 m^2 (figure 1d). In the diagnosis of hypercalcemia in patients with eGFR ≥ 60 mL/minute/1.73 m^2, they all

performed rather well (figure 1c). As judged by the Harrell's c index, unadjusted calcium was the most accurate diagnostic test in patients with eGFR < 60 mL/minute/1.73 m^2, and not inferior to any formula in patients with eGFR ≥ 60 mL/minute/1.73 m^2. The commonly used BMJ-formula ($Calcium_{adj}$ (mmol/L) = total calcium (mmol/L) + 0.02 × (40 - albumin (mmol/L), suggested in BMJ in 1977[6]), was significantly less accurate than unadjusted calcium in both eGFR groups. All calcium measures performed better in patients with eGFR < 60 mL/minute/1.73 m^2 than in those with eGFR ≥ 60 mL/minute/1.73 m^2. The ROC curve analyses partly corroborated this; however, in the diagnosis of hypercalcemia in patients with eGFR ≥ 60 mL/minute/1.73 m^2, some albumin-adjustment formulas performed slightly better than unadjusted calcium. Furthermore, the calcium measures were no better in patients with eGFR < 60 mL/minute/1.73 m^2 than in patients with eGFR ≥ 60 mL/minute/1.73 m^2. We have no explanation of this divergence between the two methods of evaluating diagnostic accuracy, other than the information loss from dichotomisation of the continuous gold standard in the ROC curve analyses. This loss of information was partly taken into consideration, as we used four different definitions of hypo- and hypercalcemia in the ROC curves analyses. We extended both definitions downwards from our reference limits of 1.18 and 1.32 mmol/L, as more recent work indicates that our reference limits may be somewhat high.[23] As expected, the area under the ROC curves increased when hypo- and hypercalcemia were defined more stringently, i.e. when the diagnoses represented more pathological cases.

Limitations of the study

Some limitations of our study should be mentioned. First, the use of pH-adjusted free calcium as a gold standard could be questioned. Although the actual concentration of free calcium in correctly sampled blood specimens should be the most relevant measure of calcium status, Thode at al. found that pH-adjusted free calcium was as useful as the actual (unadjusted) free

calcium in 183 patients with various calcium disorders.[24] Anyway, pH-adjusted free calcium was the only measure we could use, as the actual (unadjusted) free calcium was recorded in only 26 patients. Second, as no diagnostic information was available to us we do not know whether our findings are applicable to every clinical condition. However, the renal function could be estimated. The fraction of patients with an eGFR less than 60 mL/minute/1.73 m^2 was very different in ambulant and hospitalised patients (18 % versus 62 %), indicating that reduced renal function was more prevalent in hospitalised patients and/or that free calcium was more likely to be requested in hospitalised patients with reduced renal function. The relatively large number of patients with reduced renal function in the study population may be an advantage, as we know from this and other studies[16,25 26] that albumin-adjustment formulas perform differently in patients with and without renal failure. Third, we did not collect data on sodium, magnesium and parathyroid hormone. Such data could have been included for a better estimate of the albumin coefficient. However, inclusion of more variables in the same blood draw would significantly have reduced the sample size. We wanted to keep the samples size as large as possible to get a reliable estimate of the albumin coefficient.

Conclusion

We found that the diagnostic accuracy of unadjusted calcium in general is superior to that of albumin-adjusted total calcium based on formulas from literature, and even to that of locally constructed adjustment-formulas especially adapted to our dataset. Despite that many have questioned the diagnostic accuracy of albumin-adjustment formulas previously, they continue to be used in general clinical practice. We believe that the clinician should order measurement of free calcium instead of albumin-adjusted calcium in patients where total calcium is not to be trusted, as the analysis of free calcium is now widely available.

No competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical consideration

The study was carried out in full accordance with the ethical principles of the Declaration of Helsinki. According to the Norwegian Health Research Act, this type of project which uses anonymous information from local health registers, is not required to be evaluated by the Norwegian Regional Ethical Committee (REC).

Research funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Acknowledgments

None declared.

Data sharing

The full dataset is available upon request from the corresponding author (ingrid.alsos.lian@gmail.com). Informed consent for data sharing was not obtained, but the presented data are anonymised and risk of identification is low.

Contributors

IAL and AÅ both contributed to the acquisition, analysis, and interpretation of data for the work, the drafting the work or revising it critically for important intellectual content. AÅ was the main contributor in the conception and design of the work. IAL is the guarantor.

Table 1. Characteristics of the study population. Continuous variables are given as medians (2.5-97.5 percentile).

Characteristic	Inpatients	Outpatients	p - value
Number of individuals	778	5771	
Percent women	45.1	61.2	< 0.0001
Age (years)	67 (7-90)	47 (13-82)	< 0.0001
Total calcium (mmol/L)	2.22 (1.61-3.03)	2.31 (2.09-2.61)	< 0.0001
percent hypocalcemia	35.2	5.2	< 0.0001
percent hypercalcemia	10.8	5.4	< 0.0001
Free calcium (mmol/L)	1.19 (0.85-1.67)	1.23 (1.13-1.39)	< 0.0001
percent hypocalcemia	41.7	9.2	< 0.0001
percent hypercalcemia	11.3	5.3	< 0.0001
Albumin (g/L)	33 (18-45)	41 (34-46)	< 0.0001
percent hypoalbuminemia	60.0	4.9	< 0.0001
percent hyperalbuminemia	0.6	2.0	0.0063
eGFR (ml/minute/1.73m^2)	39 (7-171)	96 (19-154)	< 0.0001
percent < 60	62.3	18.2	< 0.0001
Phosphate (mmol/L)	1.18 (0.48-2.68)	1.00 (0.64-1.54)	< 0.0001

Table 2. Results of simple linear regression of total calcium against albumin, and multiple linear regression of total calcium against albumin and other relevant variables. Only the adjusted albumin coefficients were used to construct the local group-specific formulas for albumin-adjusted calcium.

	Simple linear regression	Multiple linear regression	
Subpopulation	Unadjusted albumin coefficient (95% CI)	Significant variables	Adjusted albumin coefficient (95% CI)
All with eGFR ≥ 60 mL/minute/1.73 m^2, n=5013	0.0167 (0.0158-0.0176)	Albumin, free calcium, gender, age, eGFR, phosphate	0.0126 (0.0121-0.0132)
eGFR ≥ 60 mL/minute/1.73 m^2 and albumin < 30 g/L, n=103	0.0282 (0.0158-0.0406)	Albumin, free calcium	0.0159 (0.0112-0.0206)
eGFR ≥ 60 mL/minute/1.73 m^2 and albumin ≥ 30 g/L, n=4910	0.0154 (0.0142-0.0166)	Albumin, free calcium, gender, age, eGFR, phosphate, hospitalisation status	0.0120 (0.0113-0.0128)
All with eGFR < 60 mL/minute/1.73 m^2, n=1536	0.0160 (0.0140-0.0181)	Albumin, free calcium, age, eGFR, phosphate	0.0123 (0.0113-0.0133)

Table 3. Agreement between total calcium and free calcium as measured by Harrell's c index (95% CI) in patients with eGFR above or below normal 60 mL/minute/1.73 m^2.

Adjustment formula	eGFR ≥ 60 mL/minute/1.73 m^2 (n=5013)	eGFR < 60 mL/minute/1.73 m^2 (n=1536)
None	0.749 (0.741-0.758)	0.801 (0.788-0.813)
Local*	0.749 (0.741-0.758)	0.790 (0.776-0.803)
James[20]	0.751 (0.743-0.759)	0.791 (0.777-0.804)
Orrell[4]	0.736 (0.728-0.745)	0.766 (0.751-0.781)
BMJ[6]	0.728 (0.719-0.737)	0.753 (0.738-0.769)
Thode[16]	0.726 (0.717-0.735)	0.747 (0.731-0.763)
Berry[7]	0.716 (0.707-0.725)	0.736 (0.720-0.752)
Payne[5]	0.707 (0.698-0.716)	0.723 (0.707-0.740)

*The local adjustment-formulas were constructed using three subgroup-specific albumin coefficients, see Methods

Figure legend

Figure 1. Accuracy in the diagnosis of hypocalcemia (a and b) and hypercalcemia (c and d) in patients with eGFR above (a and c) or below (b and d) 60 mL/minute/1.73 m^2, given as the area under the ROC curve for various albumin-adjustment formulas and for unadjusted calcium.

References

1. Aishah AB, Foo YN. A retrospective study of serum calcium levels in a hospital population in Malaysia. *Med J Malaysia* 1995;50(3):246-9.
2. Hastbacka J, Pettila V. Prevalence and predictive value of ionized hypocalcemia among critically ill patients. *Acta Anaesth Scand* 2003;47(10):1264-69.
3. Baird GS. Ionized calcium. *Clin Chim Acta* 2011;412(9-10):696-701.
4. Orrell DH. Albumin as an Aid to Interpretation of Serum Calcium. *Clin Chim Acta* 1971;35(2):483-9.
5. Payne RB, Little AJ, Williams RB, et al. Interpretation of Serum-Calcium in Patients with Abnormal Serum-Proteins. *BMJ* 1973;4(5893):643-46.
6. Correcting the calcium. *BMJ* 1977;1(6061):598.
7. Berry EM, Gupta MM, Turner SJ, et al. Variation in plasma calcium with induced changes in plasma specific gravity, total protein, and albumin. *BMJ* 1973;4(5893):640-3.
8. Hannan FM, Thakker RV. Investigating hypocalcaemia. *BMJ* 2013;346:f2213.
9. Ladenson JH, Lewis JW, Boyd JC. Failure of total calcium corrected for protein, albumin, and pH to correctly assess free calcium status. *J Clin Endocrinol Metab* 1978;46(6):986-93.
10. Clase CM, Norman GL, Beecroft ML, et al. Albumin-corrected calcium and ionized calcium in stable haemodialysis patients. *Nephrol Dial Transplant* 2000;15(11):1841-6.
11. Labriola L, Wallemacq P, Gulbis B, et al. The impact of the assay for measuring albumin on corrected ('adjusted') calcium concentrations. *Nephrol Dial Transplant* 2009;24(6):1834-8.
12. Rustad P, Felding P, Franzson L, et al. The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. *Scand J Clin Lab Invest* 2004;64(4):271-84.
13. Bruunhuus I, Magdal U. Kompendium 2000 - Kompendium i Laboratoriemedicin online [Edited 2008]. Available from: http://www.dskb.dk/Clubs/CommonDrive/Components/GetWWWFile.aspx?fileID=45556
14. Martensson A, Rustad P, Lund H, et al. Creatininium reference intervals for corrected methods. *Scand J Clin Lab Invest* 2004;64(4):439-41.
15. Ceriotti F, Boyd JC, Klein G, et al. Reference intervals for serum creatinine concentrations: assessment of available data for global application. *Clin Chem* 2008;54(3):559-66.
16. Jain A, Bhayana S, Vlasschaert M, et al. A formula to predict corrected calcium in haemodialysis patients. *Nephrol Dial Transplant* 2008;23(9):2884-8.
17. Pottel H, Hoste L, Dubourg L, et al. An estimated glomerular filtration rate equation for the full age spectrum. *Nephrol Dial Transplant* 2016;31(5):798-806.
18. Coresh J, Selvin E, Stevens LA, et al. Prevalence of chronic kidney disease in the United States. *JAMA* 2007;298(17):2038-47.
19. Thode J, Juul-Jorgensen B, Bhatia HM, et al. Comparison of serum total calcium, albumin-corrected total calcium, and ionized calcium in 1213 patients with suspected calcium disorders. *Scand J Clin Lab Invest* 1989;49(3):217-23.
20. James MT, Zhang J, Lyon AW, et al. Derivation and internal validation of an equation for albumin-adjusted calcium. *BMC Clin Pathol* 2008;8:12.
21. Newson RB. Interpretation of Somers' D under four simple models [Edited 2014] Available from: http://www.imperial.ac.uk/nhli/r.newson/miscdocs/intsomd1.pdf.
22. Metz CE. Basic principles of ROC analysis. *Semin Nucl Med* 1978;8(4):283-98.
23. Klaestrup E, Trydal T, Pedersen JF, et al. Reference intervals and age and gender dependency for arterial blood gases and electrolytes in adults. *Clin Chem Lab Med* 2011;49(9):1495-500.
24. Thode J, Holmegaard SN, Transbol I, et al. Adjusted ionized calcium (at pH 7.4) and actual ionized calcium (at actual pH) in capillary blood compared for clinical evaluation of patients with disorders of calcium metabolism. *Clin Chem* 1990;36(3):541-4.
25. Gauci C, Moranne O, Fouqueray B, et al. Pitfalls of measuring total blood calcium in patients with CKD. *J Am Soc Nephrol* 2008;19(8):1592-8.
26. Morton AR, Garland JS, Holden RM. Is the calcium correct? Measuring serum calcium in dialysis patients. *Semin Dial* 2010;23(3):283-9.

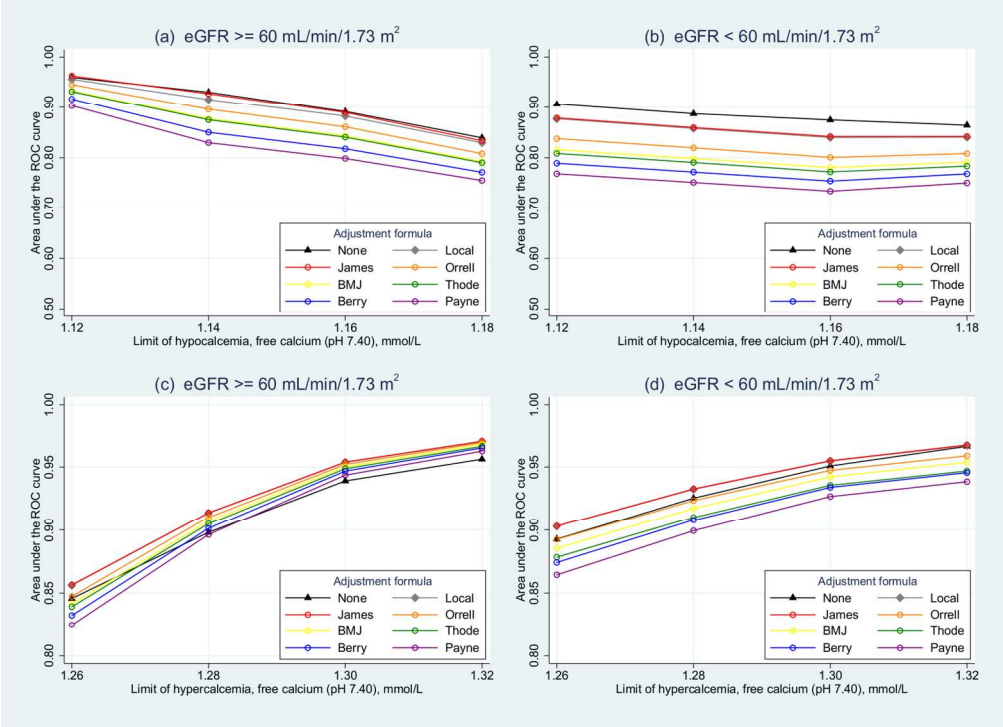


Figure 1

139x101mm (300 x 300 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2+3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Introduction, p5+6
Objectives	3	State specific objectives, including any pre-specified hypotheses	Introduction, p6
Methods			
Study design	4	Present key elements of study design early in the paper	Abstract p2+3, introduction p6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Mat and met p7+8
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Mat and met p7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Mat and met p7-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Mat and met p7-8
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	Mat and met p7, Res p11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Statistical analyses p8-10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Statistical analyses p8-10
		(*b*) Describe any methods used to examine subgroups and interactions	

		(*c*) Explain how missing data were addressed	NA, no missing data
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Mat and met p7, Res p11
		(b) Give reasons for non-participation at each stage	Res p11
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Material p6, Results p11, table 1 p19
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	Results p11-12, tables and figures p19-22
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Results p11-12, tables p19-21
		(*b*) Report category boundaries when continuous variables were categorized	Mat and met P8+9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	Discussion p13, conclusion p16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Discussion p15-16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Discussion p13-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	Discussion p14-16
Other information			

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Should total calcium be adjusted for albumin? – a retrospective observational study of laboratory data from central Norway

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017703.R3
Article Type:	Research
Date Submitted by the Author:	20-Feb-2018
Complete List of Authors:	Lian, Ingrid; St. Olavs hospital, Clinical Chemistry Åsberg , Arne; Sankt Olavs Hospital Universitetssykehuset i Trondheim, Department of Clinical Chemistry
Primary Subject Heading:	Diabetes and endocrinology
Secondary Subject Heading:	General practice / Family practice, Diagnostics
Keywords:	Calcium & bone < DIABETES & ENDOCRINOLOGY, albumin, calcium

Should total calcium be adjusted for albumin? – a retrospective observational study of laboratory data from central Norway

Ingrid Alsos Lian and Arne Åsberg

Department of Clinical Chemistry, St. Olavs hospital, 7006 Trondheim, Norway Ingrid Alsos Lian Resident in Clinical Chemistry Department of Clinical Chemistry, St. Olavs hospital, 7006 Trondheim, Norway Arne Åsberg Consultant in Clinical Chemistry

Correspondence to: ingrid.alsos.lian@gmail.com

Word count: 296/2917 | Tables: 3 | Figures: 1 | References: 26

Abstract

Objectives: Albumin-adjusted total calcium is often used as a surrogate marker for free calcium to evaluate hypo- or hypercalcemia. Many adjustment-formulas based on simple linear regression models have been published, and continue to be used in spite of questionable diagnostic accuracy. In the hope of finding a more pure albumin effect on total calcium, we used multiple linear regression models to adjust for other relevant variables. The regression coefficients of albumin were used to construct local adjustment formulas, and we tested whether the diagnostic accuracy was improved compared to previously published formulas and unadjusted calcium.

Design: A retrospective hospital laboratory data study.

Data sources: The local hospital laboratory data system.

Setting: Norway, 2006-2015.

Participants: 6549 patients above 2 years of age, where free calcium standardised at pH 7.40, total calcium, creatinine, albumin and phosphate had been analysed in a single blood draw, including hospitalised patients and patients from outpatient clinics and general practice**.**

Main outcome measures: Diagnostic accuracy by Harrell's c and ROC curve analysis, using free calcium standardised at pH 7.40 as a gold standard, in subgroups with eGFR ≥60 or <60 mL/minute/1.73m^2.

Results: In the subgroup with eGFR <60 mL/minute/1.73m^2, the Harrell's c of unadjusted total calcium (0.801) was significantly larger than those of the local formulas (0.790, p = 0.002) and the best formula taken from literature (0.791, p = 0.004). In the subgroup with eGFR ≥60 mL/minute/1.73m^2, no significant differences were found between these three formulas.

Conclusions: Our study shows that the diagnostic accuracy of *unadjusted* total calcium is

superior to several commonly used adjustment-formulas, and we suggest that the use of such formulas should be abandoned in clinical practice. If the clinician does not trust total calcium to reflect the calcium status of the patient, free calcium should be measured.

Strengths and limitations of this study

- Albumin-adjusted total calcium is often used as a surrogate marker for free calcium, to evaluate hypo- or hypercalcemia. Many adjustment-formulas have been published, and continue to be used in spite of questionable diagnostic accuracy in various patient populations.
- The diagnostic accuracy was evaluated using free calcium as the gold standard, both as a dichotomous one (with ROC curve analysis) and as a continuous gold standard (with Harrell's c index), the latter providing less loss of information, but corresponding to AUC when the gold standard is binary.
- This study includes a large group of both hospitalised and ambulant patients from a large regional hospital, representative of a broad spectrum of disease.
- No diagnostic information of the population was available, and a limited number of variables were included, as we wanted to retain a large sample size.

Introduction

Disturbances in calcium homeostasis are not uncommon in hospitalised patients,[1 2] although the exact prevalence in the general population is unknown. All calcium atoms in the body are ionized. In plasma, only 50% of the calcium ions are free to exert biological effects, whereas the rest are bound to proteins, mostly albumin, and a few percent are bound in complexes with anions like lactate and citrate.[3] The concentration of free calcium ions (hereafter named "free calcium") is closely regulated, and patients with abnormal albumin concentrations may have a normal concentration of free calcium despite abnormal concentration of total calcium. Unfortunately, free calcium is not as easily measured as total calcium, the latter being a part of routine test panels of large automatic clinical chemistry instruments. Accordingly, clinicians often try to compensate for an abnormal concentration of albumin, by calculating an albumin-adjusted calcium value, i.e. the clinician asks *"What would be this patient's concentration of total calcium if the albumin concentration were normal?"* Changes in the concentration of free calcium due to acidemia or alkalemia are disregarded in these cases. Several adjustment-formulas have been used,[4-7] and continue to be so,[8] in spite of their rather questionable diagnostic accuracy,[9] which may be worse than that of unadjusted calcium in certain populations.[10]

It is not completely clear why these adjustment-formulas perform so poorly. Some speculate that a certain formula is only valid for specific patient populations,[10] others that a certain formula may only be valid for certain analytical methods.[11] We hypothesise a more fundamental flaw – that the adjustment-formulas are based on wrongly formulated regression models. These formulas are estimated from patient populations with a range of total calcium and albumin concentrations, where the investigators have regressed the concentration of total

calcium against albumin, using simple linear regression.[4 5] The regression coefficient of albumin, usually in the range of 0.018-0.025,[6] then shows how much the total concentration of calcium is expected to change for one unit change in albumin concentration, comparing two hypothetical patients with different albumin concentrations. However, when making an albumin-adjustment we should use a coefficient that shows how much the total concentration of calcium is expected to change for one unit change in albumin concentration, when the patient's condition is *otherwise unchanged, specifically when the concentration of free calcium is unchanged*. To estimate that coefficient we have to regress the concentration of total calcium against albumin and free calcium, sex, age or whatever explanatory variable is relevant, not only albumin. Then the interpretation of the albumin coefficient gets in line with its use.

The purpose of this study was (i) to estimate regression coefficients for albumin from regression models which include the concentration of free calcium and other relevant explanatory variables, and (ii) to test whether the regression coefficients from these models yielded albumin-adjusted calcium values of better diagnostic accuracy than that of published formulas and unadjusted calcium.

Material and methods

Material

Data from our laboratory database were collected retrospectively, from January 1st 2006 to September 18th 2015 from all available patient records where the analysis of total calcium, free calcium standardised at pH 7.40, creatinine, albumin and phosphate had been performed in samples from the same blood draw (6567 patients). Only a single dataset (the oldest), from each patient was included. This included samples from both hospitalised patients and patients from outpatient clinics and general practice. No clinical information was collected. The population only included a very few critically ill patients, as free calcium in those patients were monitored using blood gas instruments in the intensive care units and the analytical results were not transferred to the laboratory information system. All samples were analysed at our laboratory at St. Olavs hospital, Trondheim, Norway, a full service acute care hospital.

Sample handling for analysis of free calcium

Almost all samples, from both hospitalised and ambulatory patients, consisted of venous blood drawn anaerobically into serum gel tubes with minimal use of stasis and muscle contraction, centrifuged with stopper in place within 1 hour and analysed within 24 hours after blood draw. Rarely, some samples from hospitalised patients or ambulatory patients may have been obtained anaerobically using blood gas syringes with electrolyte-balanced heparin. In these cases, the samples were analysed within 30 minutes after blood draw. Only samples with pH within 7.20-7.60 were accepted for analysis.

Laboratory analyses

Albumin, total calcium, creatinine and phosphate were assayed by colorimetric methods on

fully automated Modular P800 or Roche Cobas 6000 c501 instruments (Roche Diagnostics, Mannheim, Germany). The bromcreosol green (BCG) method was used for albumin. The creatinine assay was an enzymatic method calibrated against an isotope dilution mass spectrometry (IDMS) reference method. The concentration of free calcium was measured by an ionselective electrode mounted in an automated blood gas analyser (ABL 725 or ABL 825, Radiometer, Copenhagen, Denmark), and standardised at pH 7.40. Standard internal and external quality control procedures were followed for all analytical methods.

Reference ranges

Reference ranges for total calcium is 2.15-2.51 mmol/L,[12] 1.18-1.32 mmol/L for free calcium,[13] whereas our laboratory use age- and sex specific reference ranges for albumin and creatinine.[12 14 15]

Patient involvement

There was no direct patient involvement in the development, design or conduct of the study.

Statistical analysis

The dataset was divided into subgroups with estimated glomerular filtration rate (eGFR) below or above 60 mL/minute/1.73 m^2, as others have found different albumin coefficients in individuals with renal failure compared to individuals with normal renal function.[16] We used the full age spectrum (FAS) equation to calculate eGFR,[17] because the FAS equation is valid for both children (above 2 years of age) and adults. Values of eGFR above 200 mL/minute/1.73 m^2 were truncated at that level.[18] In addition, for patients with eGFR $\geq$ 60 mL/minute/1.73 m^2, we divided the dataset according to albumin concentrations below or above 30 g/L, as locally weighted scatterplot smoothing of total calcium against albumin

indicated nonlinearity overall, but linearity below and above 30 g/L. No such non-linear trend was observed for patients with eGFR < 60 mL/minute/1.73 m^2.

This procedure resulted in three subgroups. For each subgroup we created albumin-adjustment formulas: Adjusted calcium = calcium + coefficient × (40 - albumin), where the subgroup-specific albumin coefficients were estimated using multiple linear regression models with total calcium as the dependent variable and free calcium, albumin, phosphate, eGFR, gender, age and hospitalisation (or not) as the explanatory variables. We used backwards elimination until all remaining explanatory variables were statistically significant ($p < 0.05$). We also used simple linear regression with total calcium as the dependent variable and albumin as the sole explanatory variable, to estimate unadjusted albumin coefficients.

The diagnostic accuracy of the albumin-adjusted calcium coefficient calculated from the local formulas was compared to that of unadjusted total calcium and six other adjustment-formulas, taken from literature.[4-7 19 20] First, we used free calcium as a *dichotomous* gold standard to compare the diagnostic accuracies with receiver operating characteristic (ROC) curve analysis, where the patients were classified as hypocalcemic or not, and hypercalcemic or not, according to four different definitions of the diagnoses (cut-points for hypocalcemia: 1.12, 1.14, 1.16 and 1.18 mmol/L, and for hypercalcemia: 1.26, 1.29, 1.30 and 1.32 mmol/L). Second, we used free calcium as a *continuous* gold standard with Harrell's c index as a measure of diagnostic accuracy. This index is related to the area under the ROC curve. Both measures are 0.5 at no diagnostic accuracy and 1.0 at perfect diagnostic accuracy. Harrell's c takes on the same value as the area under the ROC curve when the gold standard is binary.[21] The diagnostic accuracy was studied for subgroups with eGFR below or above 60 mL/minute/1.73 m^2.

Laboratory data was extracted using SAS (version 9.2 for Windows, SAS Institute, NC, USA) and analysed using STATA (version 14.1 for Windows, StataCorp LP, TX, USA). Harrell's c index was calculated by the "somersd" procedure and differences between two indexes by the "lincom" procedure. Differences between proportions were tested by the chi-square test and differences between medians by the Mann Whitney U test. $P < 0.05$ was considered statistically significant.

Results

Clinical data

Data from a total of 6567 patients were collected, from 3895 women (59.3%) and 2672 men (40.7%). We excluded 18 patients below 2 years of age, as the FAS eGFR equation is validated for individuals aged 2 years or older.[17] Characteristics of the 6549 included patients are given in table 1. The hospitalised patients differed significantly from the out-patients in all characteristics.

Albumin coefficients

The results of simple and multiple linear regression analyses are given in table 2. The unadjusted regression coefficients of albumin were significantly different below and above 30 g/L for patients with eGFR ≥ 60 mL/minute/1.73 m^2 ($p < 0.001$). With multiple linear regression, patients with eGFR ≥ 60 mL/minute/1.73 m^2 and albumin < 30 g/L had a 32.5% higher adjusted regression coefficient than patients with eGFR ≥ 60 mL/minute/1.73 m^2 and albumin ≥ 30 g/L.

Diagnostic accuracy

The diagnostic accuracy of unadjusted calcium and albumin-adjusted calcium values calculated from the locally constructed formulas and formulas taken from literature are shown in figure 1 (ROC curve analysis) and table 3 (Harrell's c index). In patients with eGFR ≥ 60 ml/minutes/1.73 m^2, unadjusted calcium was not inferior to any albumin-adjustment formulas in diagnosing hypocalcemia (figure 1a). In patients with eGFR < 60 ml/minutes/1.73 m^2, unadjusted calcium outperformed all albumin-adjustment formulas in diagnosing hypocalcemia, independent of the definitions of hypocalcemia used in this study ($p < 0.001$ at

all definitions when compared to the formula of James et al.[20] (figure 1b)). In diagnosing hypercalcemia, unadjusted calcium performed somewhat worse than some albumin-adjustment formulas (figure 1c and 1d). When free calcium was treated as a continuous gold standard, using Harrell's c index, unadjusted calcium performed significantly better than the best calcium-adjustment formula (the formula of James et al.,[20] $p = 0.004$) and the locally constructed formulas ($p = 0.002$) in patients with eGFR < 60 ml/minutes/1.73 m^2. In patients with eGFR ≥ 60 ml/minutes/1.73 m^2 unadjusted calcium was not inferior to those formulas and ($p = 0.43$ versus the James et al. formula and $p = 0.97$ versus the locally constructed formulas) and significantly better than the other formulas ($p < 0.001$ in all cases).

Discussion

In this work, we estimated regression coefficients for albumin that reflected how much total calcium changes per unit change in albumin, adjusted for other relevant variables. To our knowledge, that has not been done before. Although theoretically sound and lower albumin coefficients were found (table 2), this procedure was nevertheless a disappointment, as the locally constructed formulas performed worse than unadjusted calcium in the subgroup of patients with eGFR < 60 mL/minutes/1.73 m^2, and no better than unadjusted calcium in the subgroup with eGFR ≥ 60 mL/minutes/1.73 m^2 (table 3). This was even more disappointing, as the local formulas were derived from and tested in the same dataset, so one would expect that their performance were positively biased. In fact, our locally constructed formulas performed very much like the formula of James et al.[20] Given that our regression coefficients for most patients are the same as or close to the value of 0.012 in the James et al. formula, equal performance is no surprise. It is more remarkable that we estimated about the same regression coefficients as James et al. when the populations, albumin methods and regression methods were different.[20] However, James et al. did not adjust for other relevant variables, so only the unadjusted albumin coefficients can be directly compared. Those coefficients were higher in our population than in the population of James et al. (table 2), probably due to different albumin methods (bromcresol green versus bromcresol purple) as well different populations. Our finding of a statistically significantly higher adjusted regression coefficient in patients with albumin ≥ 30 g/L compared to those with albumin < 30 g/L in the group with eGFR ≥ 60 mL/minute/1.73 m2 (table 2) may not be clinically significant, as our formulas did not outperform the formula of James et al. who used the same coefficient in all patient groups.

The diagnostic accuracy of albumin-adjustment formulas has been questioned previously. Almost 40 years ago, Ladenson et al.[9] compared 13 different adjustment formulas in a

population of 375 hospitalised patients and 53 controls, among them the albumin-adjustment formulas of Orrell,[4] Berry[7] and Payne[5], and found that none correlated better with free calcium than unadjusted calcium. We have found no evidence in the literature supporting that albumin-adjusted calcium is superior to unadjusted calcium in this aspect. Our study includes a relatively large group of both hospitalised and ambulant patients from a large regional hospital, representing an unselected population with a broad spectrum of disease. Compared to Ladenson et al.,[9] our findings are strengthened by a much larger study population. In addition, we have evaluated the diagnostic accuracy using free calcium both as a continuous gold standard (with Harrell's c index) and as a dichotomous one (with ROC curve analysis).

The various adjustment-formulas use different normal values of albumin. We normalised to 40 g/L, as did Payne,[5] while Orrell[4] used 34 g/L and Berry[7] 46 g/L. The choice of normal albumin value does not influence the diagnostic accuracy, because adjusted calcium = calcium + coefficient × (normal albumin - albumin) = calcium + coefficient × normal albumin - coefficient × albumin = calcium + constant + coefficient × albumin. Adding a constant to the value of a diagnostic marker does not change its diagnostic accuracy. The choice of normal albumin value does, however, influence the optimal cut-off value of albumin-adjusted calcium. Finding the optimal cut-off value could be done by ROC analysis if the prevalence of the clinical condition and the consequences of false and true positive and negative results are known,[22] but such an analysis was beyond the scope of this work.

As judged by ROC curve analysis, some of the other formulas taken from literature performed rather poorly in the diagnosis of hypocalcemia in all patients (figure 1a and 1b), and in the diagnosis of hypercalcemia in patients with eGFR < 60 mL/minute/1.73 m^2 (figure 1d). In the diagnosis of hypercalcemia in patients with eGFR ≥ 60 mL/minute/1.73 m^2, they all

performed rather well (figure 1c). As judged by the Harrell's c index, unadjusted calcium was the most accurate diagnostic test in patients with eGFR < 60 mL/minute/1.73 m^2, and not inferior to any formula in patients with eGFR ≥ 60 mL/minute/1.73 m^2. The commonly used BMJ-formula ($Calcium_{adj}$ (mmol/L) = total calcium (mmol/L) + 0.02 × (40 - albumin (mmol/L), suggested in BMJ in 1977[6]), was significantly less accurate than unadjusted calcium in both eGFR groups. All calcium measures performed better in patients with eGFR < 60 mL/minute/1.73 m^2 than in those with eGFR ≥ 60 mL/minute/1.73 m^2. The ROC curve analyses partly corroborated this; however, in the diagnosis of hypercalcemia in patients with eGFR ≥ 60 mL/minute/1.73 m^2, some albumin-adjustment formulas performed slightly better than unadjusted calcium. Furthermore, the calcium measures were no better in patients with eGFR < 60 mL/minute/1.73 m^2 than in patients with eGFR ≥ 60 mL/minute/1.73 m^2. We have no explanation of this divergence between the two methods of evaluating diagnostic accuracy, other than the information loss from dichotomisation of the continuous gold standard in the ROC curve analyses. This loss of information was partly taken into consideration, as we used four different definitions of hypo- and hypercalcemia in the ROC curves analyses. We extended both definitions downwards from our reference limits of 1.18 and 1.32 mmol/L, as more recent work indicates that our reference limits may be somewhat high.[23] As expected, the area under the ROC curves increased when hypo- and hypercalcemia were defined more stringently, i.e. when the diagnoses represented more pathological cases.

Limitations of the study

Some limitations of our study should be mentioned. First, the use of pH-adjusted free calcium as a gold standard could be questioned. Although the actual concentration of free calcium in correctly sampled blood specimens should be the most relevant measure of calcium status, Thode at al. found that pH-adjusted free calcium was as useful as the actual (unadjusted) free

calcium in 183 patients with various calcium disorders.[24] Anyway, pH-adjusted free calcium was the only measure we could use, as the actual (unadjusted) free calcium was recorded in only 26 patients. Second, as no diagnostic information was available to us we do not know whether our findings are applicable to every clinical condition. However, the renal function could be estimated. The fraction of patients with an eGFR less than 60 mL/minute/1.73 m^2 was very different in ambulant and hospitalised patients (18 % versus 62 %), indicating that reduced renal function was more prevalent in hospitalised patients and/or that free calcium was more likely to be requested in hospitalised patients with reduced renal function. The relatively large number of patients with reduced renal function in the study population may be an advantage, as we know from this and other studies[16,25 26] that albumin-adjustment formulas perform differently in patients with and without renal failure. Third, we did not collect data on sodium, magnesium and parathyroid hormone. Such data could have been included for a better estimate of the albumin coefficient. However, inclusion of more variables in the same blood draw would significantly have reduced the sample size. We wanted to keep the samples size as large as possible to get a reliable estimate of the albumin coefficient.

Conclusion

We found that the diagnostic accuracy of unadjusted calcium in general is superior to that of albumin-adjusted total calcium based on formulas from literature, and even to that of locally constructed adjustment-formulas especially adapted to our dataset. Despite that many have questioned the diagnostic accuracy of albumin-adjustment formulas previously, they continue to be used in general clinical practice. We believe that the clinician should order measurement of free calcium instead of albumin-adjusted calcium in patients where total calcium is not to be trusted, as the analysis of free calcium is now widely available.

No competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical consideration

The study was carried out in full accordance with the ethical principles of the Declaration of Helsinki. According to the Norwegian Health Research Act, this type of project which uses anonymous information from local health registers, is not required to be evaluated by the Norwegian Regional Ethical Committee (REC).

Research funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Acknowledgments

None declared.

Data sharing

The full dataset is available upon request from the corresponding author (ingrid.alsos.lian@gmail.com). Informed consent for data sharing was not obtained, but the presented data are anonymised and risk of identification is low.

Contributors

IAL and AÅ both contributed to the acquisition, analysis, and interpretation of data for the work, the drafting the work or revising it critically for important intellectual content. AÅ was the main contributor in the conception and design of the work. IAL is the guarantor.

Table 1. Characteristics of the study population. Continuous variables are given as medians (2.5-97.5 percentile).

Characteristic	Inpatients	Outpatients	p - value
Number of individuals	778	5771	
Percent women	45.1	61.2	< 0.0001
Age (years)	67 (7-90)	47 (13-82)	< 0.0001
Total calcium (mmol/L)	2.22 (1.61-3.03)	2.31 (2.09-2.61)	< 0.0001
percent hypocalcemia	35.2	5.2	< 0.0001
percent hypercalcemia	10.8	5.4	< 0.0001
Free calcium (mmol/L)	1.19 (0.85-1.67)	1.23 (1.13-1.39)	< 0.0001
percent hypocalcemia	41.7	9.2	< 0.0001
percent hypercalcemia	11.3	5.3	< 0.0001
Albumin (g/L)	33 (18-45)	41 (34-46)	< 0.0001
percent hypoalbuminemia	60.0	4.9	< 0.0001
percent hyperalbuminemia	0.6	2.0	0.0063
eGFR (ml/minute/1.73m^2)	39 (7-171)	96 (19-154)	< 0.0001
percent < 60	62.3	18.2	< 0.0001
Phosphate (mmol/L)	1.18 (0.48-2.68)	1.00 (0.64-1.54)	< 0.0001

Table 2. Results of simple linear regression of total calcium against albumin, and multiple linear regression of total calcium against albumin and other relevant variables. Only the adjusted albumin coefficients were used to construct the local group-specific formulas for albumin-adjusted calcium.

	Simple linear regression	Multiple linear regression	
Subpopulation	Unadjusted albumin coefficient (95% CI)	Significant variables	Adjusted albumin coefficient (95% CI)
All with eGFR ≥ 60 mL/minute/1.73 m^2, n=5013	0.0167 (0.0158-0.0176)	Albumin, free calcium, gender, age, eGFR, phosphate	0.0126 (0.0121-0.0132)
eGFR ≥ 60 mL/minute/1.73 m^2 and albumin < 30 g/L, n=103	0.0282 (0.0158-0.0406)	Albumin, free calcium	0.0159 (0.0112-0.0206)
eGFR ≥ 60 mL/minute/1.73 m^2 and albumin ≥ 30 g/L, n=4910	0.0154 (0.0142-0.0166)	Albumin, free calcium, gender, age, eGFR, phosphate, hospitalisation status	0.0120 (0.0113-0.0128)
All with eGFR < 60 mL/minute/1.73 m^2, n=1536	0.0160 (0.0140-0.0181)	Albumin, free calcium, age, eGFR, phosphate	0.0123 (0.0113-0.0133)

Table 3. Agreement between total calcium and free calcium as measured by Harrell's c index (95% CI) in patients with eGFR above or below normal 60 mL/minute/1.73 m^2.

Adjustment formula	eGFR ≥ 60 mL/minute/1.73 m^2 (n=5013)	eGFR < 60 mL/minute/1.73 m^2 (n=1536)
None	0.749 (0.741-0.758)	0.801 (0.788-0.813)
Local*	0.749 (0.741-0.758)	0.790 (0.776-0.803)
James[20]	0.751 (0.743-0.759)	0.791 (0.777-0.804)
Orrell[4]	0.736 (0.728-0.745)	0.766 (0.751-0.781)
BMJ[6]	0.728 (0.719-0.737)	0.753 (0.738-0.769)
Thode[16]	0.726 (0.717-0.735)	0.747 (0.731-0.763)
Berry[7]	0.716 (0.707-0.725)	0.736 (0.720-0.752)
Payne[5]	0.707 (0.698-0.716)	0.723 (0.707-0.740)

*The local adjustment-formulas were constructed using three subgroup-specific albumin coefficients, see Methods

Figure legend

Figure 1. Accuracy in the diagnosis of hypocalcemia (a and b) and hypercalcemia (c and d) in patients with eGFR above (a and c) or below (b and d) 60 mL/minute/1.73 m^2, given as the area under the ROC curve for various albumin-adjustment formulas and for unadjusted calcium.

References

1. Aishah AB, Foo YN. A retrospective study of serum calcium levels in a hospital population in Malaysia. *Med J Malaysia* 1995;50(3):246-9.
2. Hastbacka J, Pettila V. Prevalence and predictive value of ionized hypocalcemia among critically ill patients. *Acta Anaesth Scand* 2003;47(10):1264-69.
3. Baird GS. Ionized calcium. *Clin Chim Acta* 2011;412(9-10):696-701.
4. Orrell DH. Albumin as an Aid to Interpretation of Serum Calcium. *Clin Chim Acta* 1971;35(2):483-9.
5. Payne RB, Little AJ, Williams RB, et al. Interpretation of Serum-Calcium in Patients with Abnormal Serum-Proteins. *BMJ* 1973;4(5893):643-46.
6. Correcting the calcium. *BMJ* 1977;1(6061):598.
7. Berry EM, Gupta MM, Turner SJ, et al. Variation in plasma calcium with induced changes in plasma specific gravity, total protein, and albumin. *BMJ* 1973;4(5893):640-3.
8. Hannan FM, Thakker RV. Investigating hypocalcaemia. *BMJ* 2013;346:f2213.
9. Ladenson JH, Lewis JW, Boyd JC. Failure of total calcium corrected for protein, albumin, and pH to correctly assess free calcium status. *J Clin Endocrinol Metab* 1978;46(6):986-93.
10. Clase CM, Norman GL, Beecroft ML, et al. Albumin-corrected calcium and ionized calcium in stable haemodialysis patients. *Nephrol Dial Transplant* 2000;15(11):1841-6.
11. Labriola L, Wallemacq P, Gulbis B, et al. The impact of the assay for measuring albumin on corrected ('adjusted') calcium concentrations. *Nephrol Dial Transplant* 2009;24(6):1834-8.
12. Rustad P, Felding P, Franzson L, et al. The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. *Scand J Clin Lab Invest* 2004;64(4):271-84.
13. Bruunhuus I, Magdal U. Kompendium 2000 - Kompendium i Laboratoriemedicin online [Edited 2008]. Available from: http://www.dskb.dk/Clubs/CommonDrive/Components/GetWWWFile.aspx?fileID=45556
14. Martensson A, Rustad P, Lund H, et al. Creatininium reference intervals for corrected methods. *Scand J Clin Lab Invest* 2004;64(4):439-41.
15. Ceriotti F, Boyd JC, Klein G, et al. Reference intervals for serum creatinine concentrations: assessment of available data for global application. *Clin Chem* 2008;54(3):559-66.
16. Jain A, Bhayana S, Vlasschaert M, et al. A formula to predict corrected calcium in haemodialysis patients. *Nephrol Dial Transplant* 2008;23(9):2884-8.
17. Pottel H, Hoste L, Dubourg L, et al. An estimated glomerular filtration rate equation for the full age spectrum. *Nephrol Dial Transplant* 2016;31(5):798-806.
18. Coresh J, Selvin E, Stevens LA, et al. Prevalence of chronic kidney disease in the United States. *JAMA* 2007;298(17):2038-47.
19. Thode J, Juul-Jorgensen B, Bhatia HM, et al. Comparison of serum total calcium, albumin-corrected total calcium, and ionized calcium in 1213 patients with suspected calcium disorders. *Scand J Clin Lab Invest* 1989;49(3):217-23.
20. James MT, Zhang J, Lyon AW, et al. Derivation and internal validation of an equation for albumin-adjusted calcium. *BMC Clin Pathol* 2008;8:12.
21. Newson RB. Interpretation of Somers' D under four simple models [Edited 2014] Available from: http://www.imperial.ac.uk/nhli/r.newson/miscdocs/intsomd1.pdf.
22. Metz CE. Basic principles of ROC analysis. *Semin Nucl Med* 1978;8(4):283-98.
23. Klaestrup E, Trydal T, Pedersen JF, et al. Reference intervals and age and gender dependency for arterial blood gases and electrolytes in adults. *Clin Chem Lab Med* 2011;49(9):1495-500.
24. Thode J, Holmegaard SN, Transbol I, et al. Adjusted ionized calcium (at pH 7.4) and actual ionized calcium (at actual pH) in capillary blood compared for clinical evaluation of patients with disorders of calcium metabolism. *Clin Chem* 1990;36(3):541-4.
25. Gauci C, Moranne O, Fouqueray B, et al. Pitfalls of measuring total blood calcium in patients with CKD. *J Am Soc Nephrol* 2008;19(8):1592-8.
26. Morton AR, Garland JS, Holden RM. Is the calcium correct? Measuring serum calcium in dialysis patients. *Semin Dial* 2010;23(3):283-9.

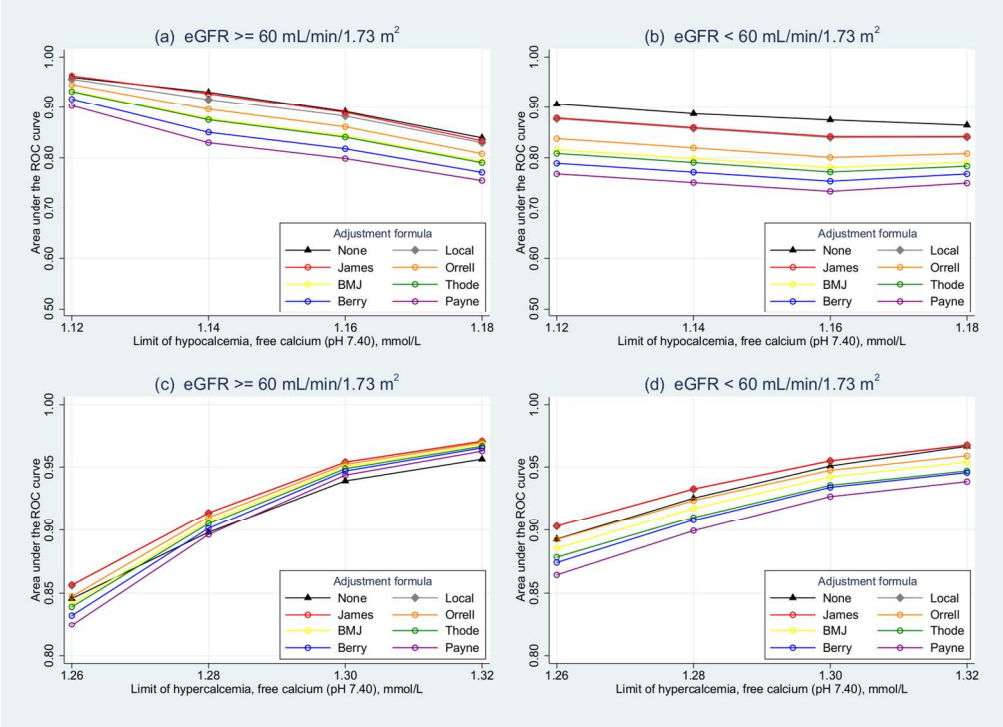


Figure 1

139x101mm (300 x 300 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2+3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Introduction, p5+6
Objectives	3	State specific objectives, including any pre-specified hypotheses	Introduction, p6
Methods			
Study design	4	Present key elements of study design early in the paper	Abstract p2+3, introduction p6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Mat and met p7+8
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Mat and met p7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Mat and met p7-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Mat and met p7-8
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	Mat and met p7, Res p11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Statistical analyses p8-10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Statistical analyses p8-10
		(*b*) Describe any methods used to examine subgroups and interactions	

		(*c*) Explain how missing data were addressed	NA, no missing data
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Mat and met p7, Res p11
		(b) Give reasons for non-participation at each stage	Res p11
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Material p6, Results p11, table 1 p19
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	Results p11-12, tables and figures p19-22
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Results p11-12, tables p19-21
		(*b*) Report category boundaries when continuous variables were categorized	Mat and met P8+9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	Discussion p13, conclusion p16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Discussion p15-16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Discussion p13-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	Discussion p14-16
Other information			

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.